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# Contents

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Effects of Cover Plants on Soil Nutrient Status and on Growth of Hevea I. Laboratory Studies on the Mineralisation of Nitrogen in Different Soil Mixtures .. .. .. .. .. .. .. 175
M. M. Guha and G. A. Watson

Poisoning Experiments with Hevea brasiliensis 1. Preliminary Trials — Destruction of Trees with 2,4,-D and 2,4,5-T .. .. .. .. .. .. 189
E. D. C. Baptiste

Poisoning Experiments with Hevea brasiliensis II. Results of a Further Tree Poisoning Experiment with 2,4,5-T .. .. .. .. .. .. .. 193
E. D. C. Baptiste and P. de Jonge

Natural Rubber Latex as Initiator for Vinyl Polymerisation .. .. .. .. .. .. .. 205
B. C. Sekhar

Control of Coptotermes curvignathus Holmgren with Chlorinated Hydrocarbons .. .. 209
A. Newsam and B. Sripathi Rao

Superior Processing Rubber Masterbatch .. .. .. 216
B. C. Sekhar and G. W. Drake

Inoculation Experiment with Fomes lignosus, Klotzch .. 223
K. P. John

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# *Effects of Cover Plants on Soil Nutrient Status and on Growth of Hevea*

# *I. Laboratory Studies on the Mineralisation of Nitrogen in Different Soil Mixtures*

M. M. GUHA and G. A. WATSON

*The results of incubation studies on the mineralisation of nitrogen in soil, and in soil mixed with ammonium sulphate, calcium carbonate and plant material, are given. Only low levels of nitrification occurred in soil alone. Applications of ammonium sulphate and calcium carbonate, at levels equivalent to 4.3 cwt/acre and 133 lb/acre respectively, had little effect on mineralisation. Calcium carbonate applied at a rate equivalent to 4 tons/acre markedly stimulated nitrification. High levels of ammonium and nitrate nitrogen were found in mixtures of soil with Centrosema pubescens, Pueraria phaseoloides and Mikania scandens material, while none was found in mixtures of soil with hevea leaves and lalang (Imperata cylindrica).*

CROP YIELDS ARE MORE UNIVERSALLY LIMITED by the level of available nitrogen in soil than by that of any other single nutrient. Available nitrogen consists of the ammonium and nitrate nitrogen contents of the soil and although there have been some reports that higher plants can assimilate nitrogen in the organic form (VIRTANEN & LINKOLA 1946, GHOSH & BURRIS 1950), it is this inorganic or mineral nitrogen which is the primary immediate source of the nutrient for all plants, other than the legumes and certain other nitrogen fixing plants. The mineral nitrogen constitutes only a small proportion of the total nitrogen in the soil; for instance, in a review of a number of experiments concerning annual agricultural crops, it has been reported that only about 3% of the total soil nitrogen was available in mineral form for uptake by the plants over a growing season of six months (BREMNER 1951). Because of this low level of available nitrogen, artificial nitrogenous fertilisers are used generally in the cultivation of annual crops with a high rate of demand for nitrogen in order to supplement the supply of available soil nitrogen. With tree crops, particularly those like hevea which grow under tropical conditions with practically a twelve month growing season, the rate of demand for nitrogen is slower and growth can be maintained at lower levels of available soil nitrogen than with most of the short season annual crops. In such instances the necessity for supplementary dressings of nitrogenous fertilisers decreases and the importance of the rate of release of available mineral nitrogen from the reserves of organic nitrogen in the soil increases.

The level of mineral soil nitrogen is governed by the well known nitrogen cycle. Plant and animal remains become incorporated in the soil and during their microbiological decomposition a certain proportion of their nitrogen content is released as ammonium nitrogen to the soil. This process, completed by the conversion of ammonium nitrogen into nitrate nitrogen, via the nitrite form, is termed the mineralisation of nitrogen. A proportion of the organic remains, including some nitrogenous compounds, is more resistant to decay, or becomes so in the process of decay, and contributes to the more stable organic matter and nitrogen reserves of the soil. Both ammonium and nitrate nitrogen can be taken up by plants and, in particular, this has recently been shown to be the case with hevea (BOLLE-JONES 1955). The

level of mineral nitrogen in the soil is governed by several factors; its rate of production is largely dependent on the degree of microbiological activity, depending as this does on the proportion of decomposable organic matter present to that of total nitrogen, and on the soil temperature, moisture, pH and other conditions. Once produced the ammonium nitrogen is subject to adsorption by the soil particles or conversion to the nitrate form. This nitrate can be quickly leached out of the soil by rainfall, or under certain conditions, a direct conversion of nitrate nitrogen to elemental nitrogen can result in a loss of gaseous nitrogen to the atmosphere.

Cultivation practices in hevea growing can greatly affect the natural mineralisation processes in the soil; clearing of the jungle in the first place exposes the soil to the sun with resultant increases in temperature and rate of mineralisation of the organic matter reserves of the soil. The use of cover plants helps to restore the equilibrium by maintaining cool and moist surface conditions, and the return of dead plant material to the soil by these plants provides a source of readily decomposable organic matter which can release nitrogen for uptake by the rubber tree. Ammonium sulphate fertiliser is generally used in hevea plantations to supplement the reserves of soil nitrogen, but it is important to understand the main characteristics of the natural nitrogen cycle in the soil and to determine in what way cultivation practices can be modified to ensure the maintenance of satisfactory levels of available soil nitrogen.

This is the first of a series of papers on this subject and gives the results of laboratory investigations which have been carried out while a series of field experiments is being laid down.

## EXPERIMENTAL

The mineralisation of nitrogen, both in soil as received from the field and in soil to which plant material or ammonium sulphate has been added, and the effect of additions of lime to the soil on this mineralisation have been studied.

Samples representing three main types of soil on which hevea is grown in Malaya were used for the work. Brief particulars of these soils, together with the results of their chemical analysis are given in Table 1.

### *Methods*

The standard bottle incubation method was used to study the mineralisation process. Three hundred grammes of soil were weighed in a basin and sufficient water was added to bring the soil moisture content to one third of the saturation capacity. The soil was mixed well and transferred into a 1 lb wide necked bottle. The bottle was corked loosely to reduce loss of moisture by evaporation and to protect the sample from contamination while allowing some movement of air, and was then incubated at a constant temperature of 30°C (±1°C). Once a week the soil was thoroughly mixed and samples were taken out for analysis. At each sampling the slight loss of moisture caused by evaporation from the soil while in the incubator was made up by addition of water. Two portions of incubated material were taken at each sampling, the amount depending on the expected content of ammonium and nitrate nitrogen, and these samples were extracted for ammonium and nitrate nitrogen separately.

For the estimation of ammonium nitrogen, the sample was shaken with an appropriate amount, depending on the ammonium nitrogen content, of N $K_2SO_4$ solution made 2N with respect to $H_2SO_4$, and filtered. The ammonium content of the filtrate was determined, in duplicate, by Conway's micro-diffusion method (Bremner & Shaw 1955).

Nitrate nitrogen was extracted with dilute calcium sulphate solution. If the extract was clear and colourless the nitrate was determined by the phenoldisulphonic acid

colorimetric method using a Spekker absorptiometer. In coloured extracts, generally obtained from samples containing high levels of decomposing organic matter, the nitrate was determined by Conway's micro-diffusion method using Devarda's alloy and magnesium oxide. Both methods had been shown in preliminary work to give identical results and their combined use lent some flexibility to laboratory arrangements.

Total nitrogen and organic carbon contents in the soil were determined by the normal micro-kjeldahl and Walkley and Black's wet digestion methods respectively. The organic nitrogen content of the soil was calculated by subtracting the mineral nitrogen content from the total nitrogen content; the resultant values have been used in all considerations of the C:N ratios of soils.

## RESULTS

### *Mineralisation of Nitrogen in Soil as Received from the Field, and the Effect of Added Ammonium Sulphate and Calcium Carbonate on this Mineralisation*

Samples of the three soils described above were incubated for seventy days. Analysis of weekly samples gave the results shown in *Figure 1*. It can be seen that, with each soil, higher levels of ammonium nitrogen than of nitrate nitrogen were found with each sampling. One marked feature, occurring with each soil, was the sharp increase in ammonium content during the first fortnight of incubation; with soil $S_3$ the level of ammonium content continued to rise steadily after the first fortnight whilst only a slight increase could be recorded with soil $S_1$, and with soil $S_2$ an actual decrease in ammonium content set in after about five to six weeks. This variation appears to be connected to the relative rates of nitrification occurring in the three soils; $S_3$ exhibited no nitrification at all over the experimental period, $S_1$ showed a slow rate of nitrification whilst a steady rate of nitrification was found with $S_2$ after the first week of incubation.

In another series of incubations ammonium sulphate was mixed with the soil at the rate of 5 mg of nitrogen per 100 gm soil, equivalent to a field application of 4.3 cwt/acre of ammonium sulphate (assuming 2 million lb soil per acre/six inches). The results given in *Figure 1* show that the basic mineralisation processes of the three soils were not altered; levels of ammonium nitrogen were higher by approximately 5 mg over the whole incubation period, and it was only with soil $S_2$ that nitrification appeared to be affected, a slight depression in the level of nitrate nitrogen produced being recorded, although the rate of nitrification was unaltered.

Application of calcium carbonate to the soil at a rate equivalent to 133 lb/acre caused no appreciable effect on the mineralisation process. When mixed in with the soil at a rate equivalent to 4 tons/acre, however, calcium carbonate caused marked effects on the mineralisation noted above. These effects are shown graphically in *Figure 2*. Comparison with *Figure 1* shows that with soils $S_1$ and $S_2$, no effect on the initial ammonification was caused by the calcium carbonate, but nitrification in these soils was so stimulated after an incubation period of two to three weeks that higher levels of nitrate nitrogen than of ammonium nitrogen were found after a further three weeks' incubation. This stimulation of the rate of nitrification ceased after another two to three weeks, when levels of ammonium and nitrate nitrogen in the soil levelled out.

Different effects were found with soil $S_3$; the initial increase in ammonium content found with the soil incubated alone was stimulated by the calcium carbonate, and vigorous nitrification was developed in the soil only after an incubation period of nine weeks. This soil showed no nitrification at all when incubated in the absence of calcium carbonate and it appears that a distinct difference exists between its nitrifying capacity and that of the other two soils.

*The Effect of Added Plant Material on the Mineralisation of Nitrogen*

The aerial portions (leaves, twigs and stems) of different cover plants which occur in hevea plantations were dried, ground and analysed (Table 2). Samples of these plant materials were mixed with samples of all three soils in the ratio of one to ten. The mixed soil and plant material was incubated as usual and samples were withdrawn for analysis at weekly intervals over a period of seventeen weeks. The results of analysis for ammonium and nitrate nitrogen in tests using material from the leguminous creepers *Centrosema pubescens* and *Pueraria phaseoloides*, and from the non-leguminous creeper *Mikania scandens* are given in *Figure 3*.

It can be seen that major differences occur between the mineralisation of the *Centrosema pubescens*/soil mixture and that of the *Mikania scandens*/soil mixture. In the former, a high rate of mineralisation produced initially very high levels of ammonium nitrogen reaching a peak equivalent to 100 mg nitrogen per 100 gm mixture, which commenced to fall after a time as vigorous nitrification developed (particularly with soils $S_1$ and $S_2$). With *Mikania scandens* mixture a much slower rate of mineralisation produced peak levels of ammonium nitrogen equivalent to only 30 mg nitrogen per 100 gm mixture. The subsequent nitrification in the *Mikania scandens* mixture was also at a lower level, except for a rather anomalous effect with soil $S_3$ in which slightly higher levels of nitrate nitrogen were produced in the *Mikania scandens* mixture than in the *Centrosema pubescens* mixture.

Ammonification in the *Pueraria phaseoloides*/soil mixtures was lower with each soil type than in the corresponding *Centrosema pubescens*/soil mixtures, particularly with soil $S_2$ and $S_3$. With soil $S_2$ and $S_3$ higher nitrification was observed in the *Pueraria phaseoloides*/soil mixtures than in the *Centrosema pubescens*/soil mixtures, while with soil $S_1$ there was little difference between the two mixtures.

Incubations of mixtures of lalang (*Imperata cylindrica*) and senescent hevea leaves with soils were also carried out, but in these instances no mineral nitrogen at all was produced during incubation.

The carbon and nitrogen contents and the C:N ratios of the untreated soils before incubation are given in Table 1, while those of the samples after incubation with or without applied ammonium sulphate and/or calcium carbonate are shown in Table 3. Though the three soils differ considerably as to their organic carbon and nitrogen contents, the C:N ratios both before and after incubation varied only between 9.00 and 11.9. This similarity, together with the low rate of decomposition, leads one to suppose that the soils contained only low reserves of decomposable organic matter.

The pH values of the soils alone, of soils treated with ammonium sulphate equivalent to 4.3 cwt/acre and/or calcium carbonate equivalent to 4 tons/acre, and of the soil/plant material mixtures were determined both before and after incubation and are shown in Table 3. The C:N ratios of the plant materials alone are given in Table 2 and those of plant/soil mixtures are included in Table 3. The latter also summarises the total nitrogen mineralised, in actual amount and as a percentage of the original nitrogen, calculated from the total amount of ammonium and nitrate nitrogen produced during incubation of these mixed samples. It can be seen that the mineral nitrogen produced during incubation of *Mikania scandens* mixtures is much lower, both in amount and in percentage of the original organic nitrogen, than that produced in *Pueraria phaseoloides* or *Centrosema pubescens* mixtures.

## DISCUSSION

Three main points that arise from the above work call for discussion. They are the variation in nitrifying capacities of the three soil types used, and the effects of added calcium carbonate and plant materials respectively on the mineralisation process.

The ammonification and nitrification processes in the three untreated soils during incubation are typical of soils of high acidity and moderate organic matter content (PAUL & SHARIFF 1954, STOBE 1952, DUCHAUFOUR 1954). A comparatively high level of ammonification was followed by the development of only a low level of nitrification. It appears possible that even within the small range of pH values covered by these three soils, the degree of nitrification was related to the soil reaction — the soil with the lowest pH value ($S_3$) exhibiting the lowest rate of nitrification — and that the slow rates of nitrification in these soils, as compared to that in nearly neutral soils (ACHARYA & JAIN 1954), was the direct result of their low pH values. A greater number of soils with differing acidities would need to be studied before a definite conclusion on the above could be made, however. Doubtless the environmental conditions of the soils in the field will have exerted some effect; for instance soil $S_3$ was taken from shaded conditions under mature hevea whilst soils $S_1$ and $S_2$ came from replanting sites that had been exposed to the sun for some time, with possible consequences on their microbial population.

Though the organic nitrogen contents of the three untreated soils differed considerably, the amounts of nitrogen mineralised during incubation were all low and similar and were not related to the respective C:N ratios determined both before and after incubation. This is not surprising since variation in the histories of the soils will have produced differences in the soil organic matter such that the values of the C:N ratio at which only low values of mineralisation of nitrogen are found will vary from soil to soil (ANDERSON & BYERS 1934, KOHNLEIN 1955, ALBAREDA 1955).

The addition of ammonium sulphate to the soils only slightly affected the soil reaction (Table 3) and no significant effects on the mineralisation process were noted. The addition of calcium carbonate to the soil at a rate equivalent to 133 lb/acre, intended to affect only the nutrition of possible nitrifiers (STEVENSON & CHASE 1953) without appreciable change in pH value of the medium, caused no significant increase in the rate of nitrification. However, the addition of calcium carbonate at a level equivalent to 4 tons/acre caused major increases in both pH values and in nitrification, and it would appear likely that this is an instance where a decrease in acidity has stimulated development of the nitrifying population (MULDER 1950, CORNFIELD 1952, 1953, MAZILKIN 1954). The stimulation of nitrification took longer to develop with soil $S_3$ than with the other two soils. This may be related to the absence of nitrification found in the incubation of this soil when untreated. A microbiological examination of the soils might show that soil $S_3$ possessed an initially much smaller population of nitrifiers than did the other two soils.

In further tests, additions of calcium carbonate equivalent to a rate of 9 tons/acre caused no additional increase in nitrification over that produced by the dressing of 4 tons/acre; this agrees with other work showing that increases in pH values of over 6.5 by additions of calcium carbonate did not result in further increases in nitrification (CORNFIELD 1952).

In connection with this effect of pH on nitrification, it has been shown that fungi are predominant in the microbiological population of Malayan acid soils (CORBETT 1934); fungi are known to be effective ammonifiers (WAKSMAN 1952) and this combination of facts may explain why, in the untreated soils, ammonification was at a higher level than nitrification.

Admixtures of *Pueraria phaseoloides*, *Centrosema pubescens* and *Mikania scandens* to all three soils resulted in high rates of ammonification and subsequent nitrification, with *Pueraria phaseoloides* having the greatest effect and *Mikania scandens* the least. The variation in the amounts of nitrogen mineralised must have been dependent to some extent on the nature and ease of decomposition of the different plant materials and not primarily on their nitrogen contents nor on their C:N ratios, for the differences

between the three plant materials with respect to these factors seem hardly large enough to explain adequately the comparatively low level of mineralisation shown by the *Mikania scandens* (Table 3). Neither senescent rubber leaves nor lalang with C:N ratios respectively of 27.9 and 61.2 showed any mineralisation at all when mixed with soil, in the time covered by the experiment; this fact is in line with the theory that only material with a C:N ratio of 20:1 or lower can, in general, directly provide mineral nitrogen (HARMSEN & VAN SCHREVEN 1955).

The addition of some plant materials caused even greater increases in nitrification than that caused by the addition of calcium carbonate. Some intermediate determinations showed that, during the initial ammonification, the pH values of incubated samples increased until nitrification commenced, but fell again with the production of nitrate. The ammonification and nitrification effects in the soil/plant material mixtures are reflected in the change of pH values shown in Table 3. It is possible that nitrification was initiated in the soil/plant material mixtures by the decrease in acidity consequent on ammonification; the presence of ammonium nitrogen in itself was not sufficient to stimulate nitrification, for the addition of ammonium sulphate to soil had no effect on nitrification nor on pH value. This may explain why in some acid soils ammonium nitrogen from ammonium hydroxide or anhydrous ammonia is more rapidly nitrified than that from ammonium sulphate (ENO & BLUE 1954, BIRECKA et alii 1954, ANDERSON & PURVIS 1955).

It can be tentatively concluded from this preliminary work that, in bare soil containing only low levels of easily decomposable organic matter, the mineralisation of soil nitrogen is likely to consist largely of ammonification, only a low level of nitrification being attained. The rate of nitrification can be stimulated by raising the pH values of the soil by addition of calcium carbonate while the addition of large quantities of decomposable organic matter will stimulate both ammonification and nitrification. In young and mature plantations where such organic matter is returned to the soil as litter from cover plants comparatively high rates of ammonification and nitrification may exist. In mature plantations where hevea leaves form the principal leaf litter, it seems unlikely that rapid mineralisation of the leaf nitrogen content can occur.

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TABLE 1. CHEMICAL ANALYSIS OF SOIL SAMPLES

*Results are calculated on oven dry basis*

| Soil | Site | pH | Ammonium nitrogen p.p.m. | Nitrate nitrogen p.p.m. | Organic nitrogen % | Organic carbon % | Organic carbon / nitrogen | Exchangeable cations (m.eq. per 100 gm soil) Ca | Mg | K |
|---|---|---|---|---|---|---|---|---|---|---|
| $S_1$ — a coastal alluvial clay-loam (Selangor series, Owen 1951) | Old hevea plantation cleared for replanting | 5.09 | 30 | 35 | 0.295 | 2.73 | 9.25 | 3.08 | 3.17 | 0.96 |
| $S_2$ — a clay-loam containing a high proportion of coarse quartz granules (Rengam series soil) | Young hevea plantation; undulating ground lightly covered by weeds and grasses | 5.39 | 39 | 5 | 0.166 | 1.92 | 11.57 | 1.19 | 0.06 | 0.32 |
| $S_3$ — a sandy loam derived from sandstone. (Kedah series) | Mature hevea plantation; undulating ground supporting indigenous mixed cover plants | 4.59 | trace | trace | 0.139 | 1.25 | 8.99 | 0.08 | 0.07 | 0.10 |

TABLE 2. CHEMICAL ANALYSIS OF PLANT MATERIALS

*Results are calculated as percentages on oven dry basis*

| Plant material | Carbon | Nitrogen | Carbon/Nitrogen |
|---|---|---|---|
| *Pueraria phascoloides* | 41.70 | 2.91 | 14.3 |
| *Mikania scandens* | 38.76 | 2.44 | 15.9 |
| *Centrosema pubescens* | 40.32 | 3.53 | 11.4 |
| *Imperata cylindrica* (lalang) | 45.28 | 0.74 | 61.2 |
| Senescent hevea leaves | 42.40 | 1.52 | 27.9 |

TABLE 3. CHANGES IN ORGANIC CARBON, NITROGEN, CARBON/NITROGEN RATIOS AND pH VALUES DURING INCUBATION

*Carbon and nitrogen results are expressed as percentages on oven-dry basis.*

| Value<br>I = before incubation<br>II = after incubation<br>III = I – II | | Soil alone | Soil + ammonium sulphate | Soil + calcium carbonate | Soil + ammonium sulphate + calcium carbonate | Soil + *P. phaseoloides* | Soil + *M. scandens* | Soil + *C. pubescens* |
|---|---|---|---|---|---|---|---|---|
| *Soil* $S_1$ | | | | | | | | |
| Organic carbon | I | 2.73 | 2.73 | 2.73 | 2.73 | 6.31 | 5.93 | 6.11 |
| | II | 2.74 | 2.70 | 2.79 | 2.71 | 4.23 | 4.62 | 4.43 |
| | III | + 0.01 | – 0.03 | + 0.06 | – 0.02 | – 2.08 | – 1.31 | – 1.68 |
| Organic nitrogen | I | 0.295 | 0.295 | 0.295 | 0.295 | 0.531 | 0.475 | 0.587 |
| | II | 0.281 | 0.285 | 0.270 | 0.270 | 0.476 | 0.433 | 0.457 |
| | III | – 0.014 | – 0.010 | – 0.025 | – 0.025 | – 0.055 | – 0.042 | – 0.130 |
| Carbon/ nitrogen ratio | I | 9.25 | 9.25 | 9.25 | 9.25 | 11.88 | 12.48 | 10.40 |
| | II | 9.75 | 9.47 | 10.33 | 10.04 | 8.89 | 10.67 | 9.69 |
| | III | + 0.50 | + 0.22 | + 1.08 | + 0.79 | – 2.99 | – 1.81 | – 0.71 |
| Amount and percentage of N mineralised | | 0.012 (4.1%) | 0.012 (4.1%) | 0.017 (5.8%) | 0.016 (5.4%) | 0.075 (14.1%) | 0.025 (5.3%) | 0.100 (17.0%) |
| pH | I | 5.09 | 4.75 | 5.83 | 5.78 | 5.32 | 4.38 | 5.06 |
| | II | 4.40 | 4.34 | 4.74 | 4.91 | 4.60 | 5.62 | 4.51 |
| | III | – 0.69 | – 0.41 | – 1.09 | – 0.87 | – 0.72 | + 1.24 | – 0.55 |

*Soil $S_2$*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Organic carbon | I | 1.92 | 1.92 | 1.92 | 1.92 | 5.43 | 5.07 | 5.26 |
| | II | 1.85 | 1.78 | 1.67 | 1.76 | 3.44 | 3.37 | 3.67 |
| | III | − 0.07 | − 0.14 | − 0.25 | − 0.16 | − 1.99 | − 1.70 | − 1.59 |
| Organic nitrogen | I | 0.166 | 0.166 | 0.166 | 0.166 | 0.401 | 0.349 | 0.449 |
| | II | 0.156 | 0.152 | 0.138 | 0.151 | 0.379 | 0.336 | 0.355 |
| | III | − 0.010 | − 0.014 | − 0.028 | − 0.015 | − 0.022 | − 0.013 | − 0.094 |
| Carbon/ nitrogen ratio | I | 11.57 | 11.57 | 11.57 | 11.57 | 13.54 | 14.53 | 11.71 |
| | II | 11.86 | 11.71 | 12.10 | 11.67 | 9.08 | 10.03 | 10.34 |
| | III | + 0.29 | + 0.14 | + 0.53 | + 0.10 | − 4.46 | − 4.50 | − 1.37 |
| Amount and percentage of nitrogen mineralised | | 0.013 (7.8%) | 0.012 (7.2%) | 0.015 (9.0%) | 0.012 (7.2%) | 0.060 (15.0% | 0.013 (3.7% | 0.040 (8.9%) |
| pH | I | 5.39 | 5.31 | 7.16 | 7.28 | 5.27 | 5.35 | 5.44 |
| | II | 4.70 | 4.52 | 6.65 | 6.10 | 4.65 | 5.50 | 5.32 |
| | III | − 0.69 | − 0.79 | − 0.51 | − 1.18 | − 0.62 | + 0.15 | − 0.12 |

*Soil $S_3$*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Organic carbon | I | 1.25 | 1.25 | 1.25 | 1.25 | 4.77 | 4.42 | 4.61 |
| | II | 1.37 | 1.31 | 1.28 | 1.37 | 3.06 | 2.84 | 3.04 |
| | III | + 0.12 | + 0.06 | + 0.03 | + 0.12 | − 1.71 | − 1.58 | − 1.57 |
| Organic nitrogen | I | 0.139 | 0.139 | 0.139 | 0.139 | 0.373 | 0.322 | 0.425 |
| | II | 0.126 | 0.123 | 0.121 | 0.122 | 0.318 | 0.294 | 0.238 |
| | III | − 0.013 | − 0.016 | − 0.018 | − 0.017 | − 0.055 | − 0.028 | − 0.187 |
| Carbon/ nitrogen ratio | I | 8.99 | 8.99 | 8.99 | 8.99 | 12.78 | 13.73 | 10.85 |
| | II | 10.87 | 10.65 | 10.58 | 11.23 | 9.62 | 9.66 | 12.77 |
| | III | + 1.88 | + 1.66 | + 1.59 | + 2.24 | − 3.16 | − 4.07 | + 1.92 |
| Amount and percentage of nitrogen mineralised | | 0.014 (10.17%) | 0.013 (9.4%) | 0.015 (10.4%) | 0.014 (10.1%) | 0.070 (18.8%) | 0.008 (2.5%) | 0.055 (12.9%) |
| pH | I | 4.59 | 4.70 | 6.91 | 7.11 | 4.77 | 5.43 | 5.15 |
| | II | 5.72 | 5.05 | 5.40 | 5.15 | 5.01 | 5.75 | 5.90 |
| | III | + 1.13 | + 0.35 | − 1.51 | − 1.96 | + 0.24 | + 0.32 | + 0.75 |

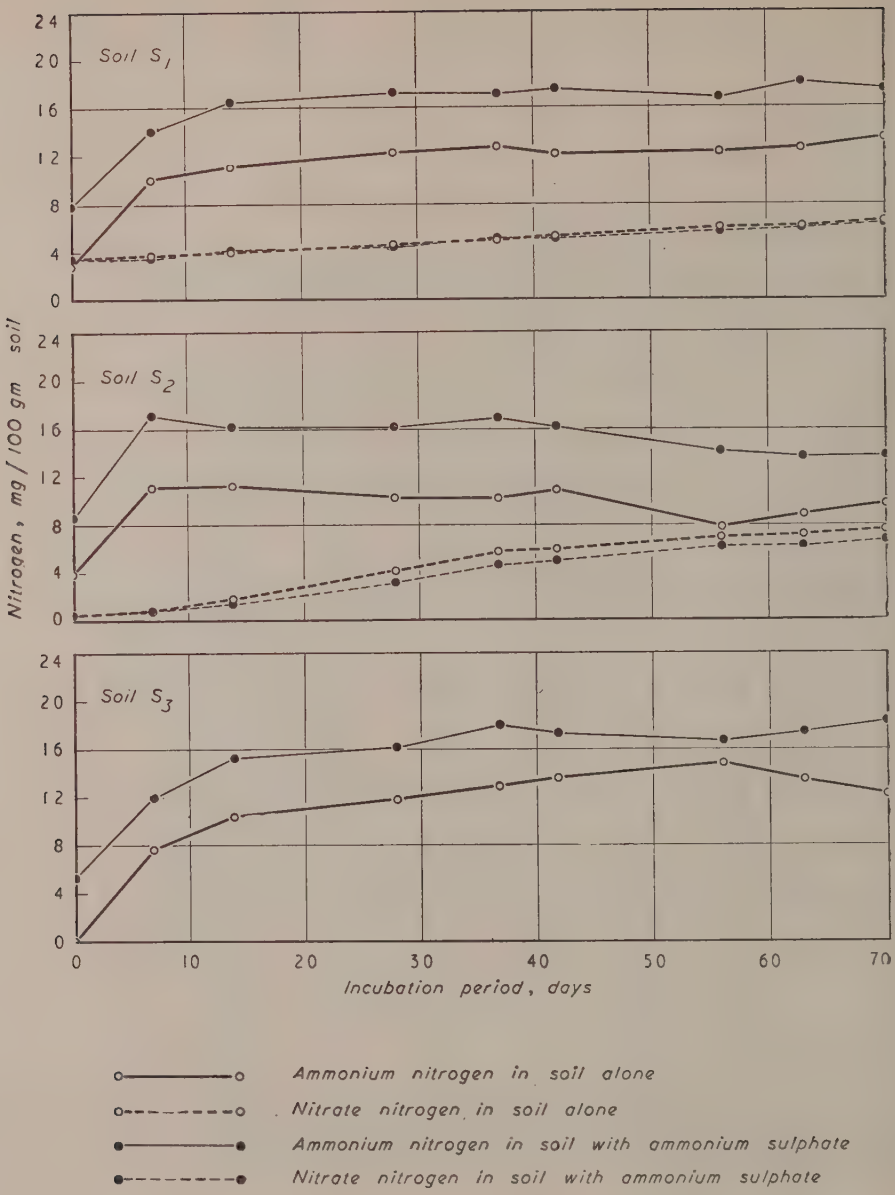
Soil $S_1$
Soil $S_2$
Soil $S_3$
Nitrogen, mg / 100 gm soil
Incubation period, days
Ammonium nitrogen in soil alone
Nitrate nitrogen in soil alone
Ammonium nitrogen in soil with ammonium sulphate
Nitrate nitrogen in soil with ammonium sulphate

*Figure 1.* Mineralisation of nitrogen in soil alone and in soil mixed with ammonium sulphate.

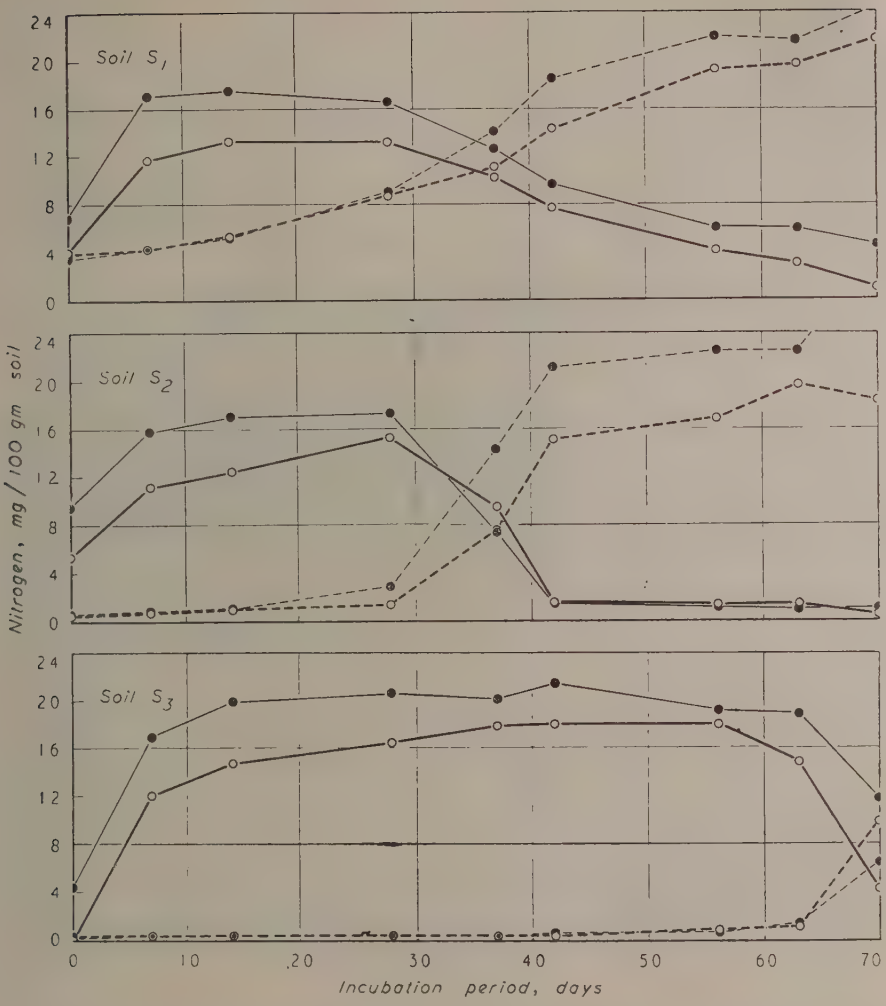


*Figure 2.* Mineralisation of nitrogen in soil mixed with calcium carbonate, and with calcium carbonate and ammonium sulphate.

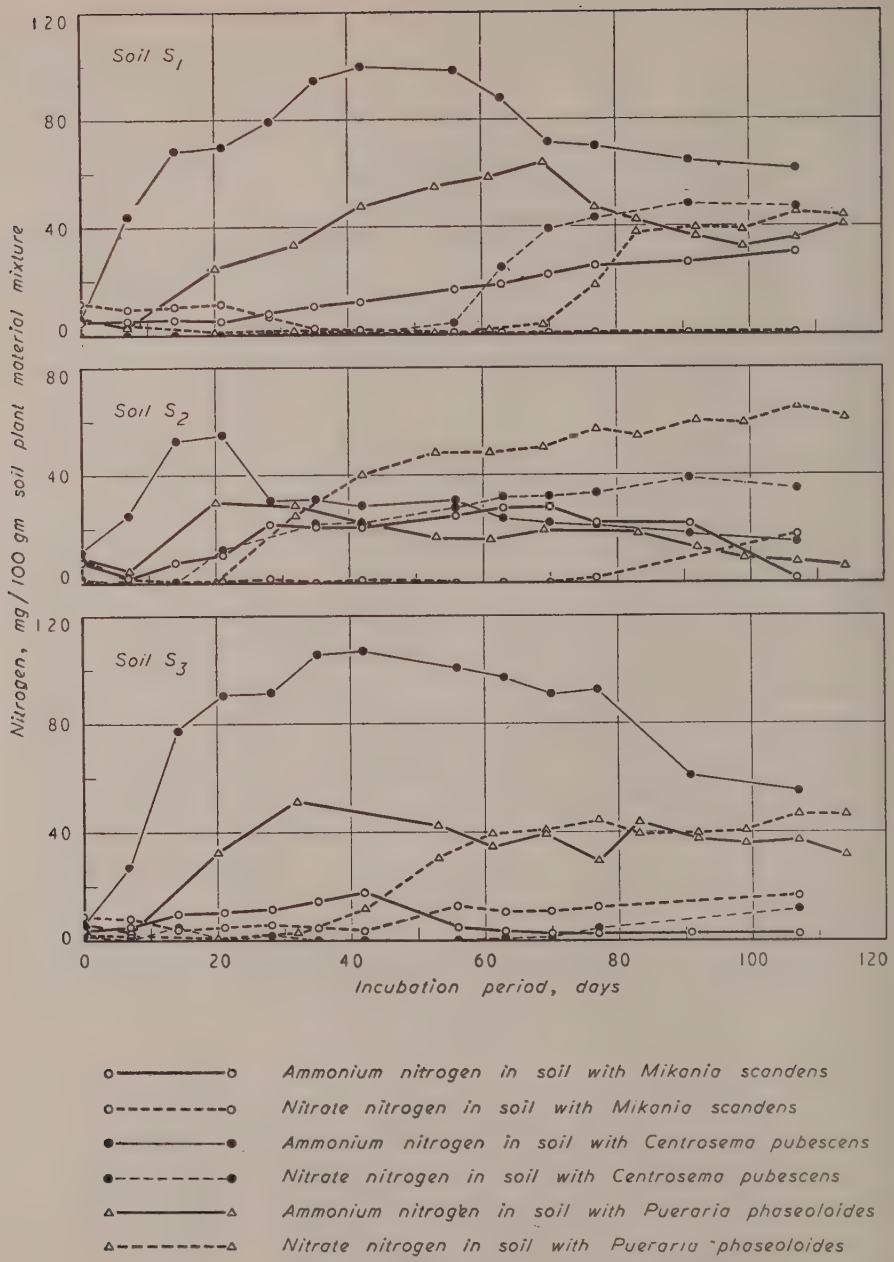


Figure 3. Mineralisation of nitrogen in soil mixed with Mikania scandens, Centrosema pubescens and Pueraria phaseoloides material.

# *Poisoning Experiments with Hevea brasiliensis*
# *I. Preliminary Trials—Destruction of Trees with 2, 4,-D and 2, 4, 5-T*

E. D. C. BAPTISTE*

*A brief review is given of experiments on tree poisoning. The results of these early experiments led to the development and large scale use of sodium arsenite and later the normal butyl ester of 2, 4, 5-T as tree poisons.*

THE METHOD OF TREE POISONING recommended for use on estates before and immediately after the war consisted of cutting a horizontal frill one to two inches deep, girdling the trunk, and pouring into the frill a solution of sodium arsenite at a concentration of 5 lb of the chemical per gallon of water. This method, which has proved very successful under estate conditions, was evolved as a result of early work on tree poisoning commenced at the R.R.I. and continued in cooperation with the Forest Research Institute, Kepong, from 1936 to 1939. Earlier methods tried, which proved either unreliable or uneconomic, consisted in applying solutions or crystals of a wide range of plant poisons in auger holes, notches or frills made in the tree trunk or in spraying or painting the tree trunk with similar solutions[1].

The frill girdling method has been superseded to some extent by the simpler and cheaper method of ring barking, which consists in removing a ring of bark about eight inches wide and immediately painting the exposed wood surface with a thin paste containing 5 lb of sodium arsenite and 6 oz tapioca starch to a gallon of water[2]. Sodium arsenite, however, is a dangerous poison; its use in Malaya is governed by the Poisons (sodium arsenite) Ordinance 1949 which defines conditions for purchase, storage and use in agricultural operations, with which small estate owners and especially smallholders, cannot usually comply. Experiments have therefore been carried out to discover an effective tree poison which is less dangerous in use than sodium arsenite and which can be used by smallholders or other persons unable to comply with the regulations governing the use of sodium arsenite.

## EXPERIMENTAL

Early trials carried out in October 1953 with formulations of 2, 4-dichlorophenoxy acetic acid and 2, 4, 5-trichlorophenoxy acetic acid as tree poisons showed considerable promise. The normal butyl esters of 2, 4-D and 2, 4, 5-T in Diesoline and the water-soluble amine of 2, 4, 5-T in water were applied to the trees in a frill girdle or on a

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* Experimental work on the use of 2, 4, 5-T in the killing of rubber trees prior to replanting was initiated by Dr E. D. C. Baptiste in 1953. Subsequent work has been carried out by Mr P. de Jonge, in cooperation with Dr Baptiste until June 1955 when it was taken over by Mr de Jonge. Dr E. D. C. Baptiste became Director of the Rubber Research Institute of Ceylon in January 1956.

COMMUNICATION 316

ring barked portion of tree trunk at concentrations of 1%, 5% and 10%. One pint of the solution was introduced into each frill girdle and one quarter pint was applied to the exposed wood surface after ring barking.

The overall results after six months showed about the same incidence of kills, approximately 90% with the normal butyl esters of 2, 4-D and 2, 4, 5-T, and only 50% of kills with the water-soluble amine of 2, 4, 5-T. There was a slight advantage of the frill girdling method of application over that of the ring barking method.

The best results were obtained with the normal butyl ester of 2, 4, 5-T which gave 100% kills when used at concentrations of 5% and 10% in Diesoline, followed by the normal butyl ester of 2, 4-D, which gave 84% kills at a concentration of 5% and 100% kills at a concentration of 10%. The water-soluble amine of 2, 4, 5-T was ineffective when used at a concentration of 1% but gave about 75% kills at concentrations of 5% and 10%.

Preliminary experiments with applications of the normal butyl ester of 2, 4, 5-T to the bark at the base of a small number of trees showed such promising results[3] that a series of experiments were set up on old rubber trees during 1954 in collaboration with the R.R.I. Smallholders' Advisory Service. The normal butyl ester of 2, 4, 5-T was used at concentrations of 5% and 10% in Diesoline in experiment *A* and at a concentration of 2% in experiment *B*. The results of experiment *A*, observed six months after treatment are summarised in Table 1.

Table 1. Effects of Three Methods of Application of 2, 4, 5-T

| Ring barking at breast height with 10 inches wide ring | | Painting of renewing bark on tapping panel | | Painting basal portion of tree trunk | | | |
|---|---|---|---|---|---|---|---|
| | | | | 10 in. | 15 in. | 10 in. | 15 in |
| Concentration | 5% 10% | 5% | 10% | 5% | | 10% | |
| % kill | 99 100 | 0 | 0 | 100 | 100 | 100 | 100 |
| Average time in months for trees to die | 3 2½ | — | — | 2½ | 2 | 2½ | 1½ |
| Amount used per tree (fl oz) | 2 2½ | 1½ | 2 | 5 | 6 | 5 | 6 |
| Average girth (inches) at a height of 50 inches | 26 36 | 27 | 47 | 27 | 27 | 38 | 27 |

The tree poison has been equally effective whether applied to a ring barked portion of the stem or directly on to the bark near the base of the tree. Application to the tapped panel on half the circumference of the trunk has been quite ineffective.

In experiment B only a 2% solution of the normal butyl ester of 2, 4, 5-T in Diesoline has been used. The average girth of the old seedling trees was 30 inches at 50 inches from the ground. Six months after basal application of the solution to a band 15 inches wide by means of a paint brush 88% kills were recorded at an average time of three months from treatment.

In a further experiment carried out on boundary row trees of Field 23 on the Rubber Research Institute Experiment Station the following treatments were applied, using a paint brush, to the bark from ground level up to a height of 15 inches[1].

*A* 2% solution of n-butyl ester of 2, 4, 5-T in Diesoline

*B* 5% solution of n-butyl ester of 2, 4, 5-T in Diesoline

*C* 10% solution of n-butyl ester of 2, 4, 5-T in Diesoline

*D* 5% solution of n-butyl ester of 2, 4, D in Diesoline

*E* 5 lb per gallon of sodium arsenite thickened with starch

*F* 10 lb per gallon of sodium arsenite thickened with starch

The results six months after treatment are summarised in Table 2.

Table 2. Effects of Six Tree Treatments, Six Months After Application

| Treatment | Average girth of trees at 15 inches | Percentage of kills | Average time (months) for trees to die |
|---|---|---|---|
| *A* | 59.7 inches | 75 | 6 |
| *B* | 58.3 inches | 92 | 4½ |
| *C* | 63.7 inches | 100 | 4½ |
| *D* | 58.4 inches | 78 | 5 |
| *E* | 55.1 inches | 0 | — |
| *F* | 59.1 inches | 0 | — |

The effect of the 2, 4, 5-T treatments was reflected in a short period of greatly increased yield and it appeared to be worth while to investigate the possibility of a slower kill combined with continued tapping on both virgin and renewed bark. It was also observed that bark development in the treated region appeared to be stimulated markedly, the bark becoming soft and spongy with a notable increase in thickness.

## SUMMARY

Methods of tree poisoning are briefly reviewed. Experiments are described and it is shown that the normal butyl ester of 2, 4, 5-trichlorophenoxy acetic acid (2, 4, 5-T) in

gas oil (diesel oil) is an effective tree poison when applied in 5% concentration in Diesoline, by spray or by brush, to the bark at the base of rubber trees from ground level up to a height of about fifteen inches.

Most of the treated trees were killed during the first six months after treatment; and the treatment results in greatly increased yields and in a large increase in bark thickness at the site of application.

*I have to record my indebtedness to Mr L. R. Davidson, Smallholders' Advisory Officer, for his generous cooperation; to Mr M. I. Nayar, Senior Field Assistant and to Mr Fong Fook Lam, Laboratory Assistant, Botanical Division, for their assistance in applying the treatments, taking regular observations, and in summarising the results.*

*Botanical Division*
*Rubber Research Institute of Malaya*
*Kuala Lumpur*

*Accepted February*

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[2]RRIM, *Plant. Bull.* 1(1952)3.

[3]RRIM, *J. Rubb. Res. Inst. Malaya* 14(1953)179.

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# *Poisoning Experiments with Hevea brasiliensis II. Results of a Further Tree Poisoning Experiment with 2, 4, 5-T*

E. D. C. BAPTISTE and P. DE JONGE

*An experiment is described in which three different grades of gas oil confounded with two concentrations of 2, 4, 5-trichlorophenoxy acetic acid have been compared as poisons of old Hevea brasiliensis trees.*

*The effect of these mixtures on rate of death of trunks, branches and roots is discussed together with the effect on the yield.*

THE PRACTICAL VALUE OF 2, 4, 5-T as a tree poison suggested in Part I has been subjected to further investigation in an experiment carried out on the R.R.I. Experiment Station. Two concentrations of the normal butyl ester of 2, 4, 5-T have been compared in three different oil carriers on twentyfive-year-old well grown unselected seedling trees. The following oils were used containing 0, 2½% or 5% acid-equivalent of 2, 4, 5-T.

1 Shell Diesoline (low viscosity)

2 Shell Diesel Fuel (medium viscosity)

3 Shell Fuel Oil (high viscosity)

Furthermore, a comparison was made between normal S/2.d/2.100% tapping in renewed bark and V/2.d/2.100% tapping in an upward direction in virgin bark on both poisoned and control trees. Tapping of poisoned trees was continued until the cuts dried out.

The experimental formulations were applied to a full circumference band at the base of the trees from ground level to a height of fifteen inches. The downward tapped cuts, therefore, moved toward the treated strip of bark, whereas the upward tapped cuts moved away from it.

The average quantity applied per tree was 220 ml for all formulations. This high dose was necessitated by the large girth of the trees (66.2 inches) at the place of application, and works out at approximately 0.22 ml per square inch of bark.

COMMUNICATION 317

The following thirteen treatments have been compared:

| | | |
|---|---|---|
| *A* | Diesoline | Normal S/2.d/2.100% tapping in renewed bark |
| *B* | Diesel fuel | |
| *C* | Fuel oil | |
| *D* | Diesoline + 2½% 2, 4, 5-T | |
| *E* | Diesel fuel + 2½% 2, 4, 5-T | |
| *F* | Fuel oil + 2½% 2, 4, 5-T | |
| *G* | Diesoline + 5% 2, 4, 5-T | |
| *H* | Diesel fuel + 5% 2, 4, 5-T | |
| *J* | Fuel oil + 5% 2, 4, 5-T | |
| *K* | Diesoline + 2½% 2, 4, 5-T | Upward V/2.d/2.100% tapping in renewed bark |
| *L* | Diesoline + 5% 2, 4, 5-T | |
| *M* | Control | |
| *O* | Control | Normal S/2.d/2.100% tapping in renewed bark |

*Design of experiment*

A suitable design to compare such a large number of treatments with a reasonable degree of accuracy without using an unduly large acreage is the 'tree-plot-design', in which each tree is considered as a treatment plot. Earlier experiments on yield stimulation had shown that 30 trees per treatment gives a satisfactory basis for judgement of yield performance. The area available allowed for 35 trees per treatment. Some planning was given to 'randomisation', by the introduction of 35 theoretical blocks of 13 trees each. Trees within each block were, on the average, of nearly similar yield capacity, as judged by two months' yield recording of individual trees; and, by further arrangements, each treatment contained approximately the same number of vigorous, moderate and poorly grown trees. Randomisation of treatments was done within blocks.

*Recording*

The yield was recorded by coagulation of the latex in the cup on all tapping days. Individual tree yields had been recorded over a period of two months before the treatments were applied and the yield recording was continued until the trees ceased to yield.

The girth of each tree was measured around the centre of the bark strip to be treated, i.e. at approximately 7½ inches from ground level. (See Table I).

The importance of taking bark thickness measurements at a point on the site of applications of the poison before the treatments were applied was realised too late. Measurements were taken one month later at 5 inches above the treated strip of bark at the North, East, West and South positions on each tree. They showed an increase of bark thickness when 2, 4, 5-T was applied in Diesoline and diesel fuel, but not when it was applied in the highly viscous fuel oil (see Table I). However, the

TABLE 1. GIRTH, BARK THICKNESS AND NUMBER OF DEAD TREES IN EACH TREATMENT (35 TREES)

| Treatment | Mean girth in inches | *Mean bark thickness in mm | Trees dead after one year | |
|---|---|---|---|---|
| | | | number | percentage |
| *A* Diesoline | 67.0 | 7.9 | Nil | — |
| *B* Diesel fuel | 69.7 | 8.4 | Nil | — |
| *C* Fuel oil | 67.9 | 7.7 | Nil | — |
| *D* Diesoline + 2½% 2, 4, 5-T | 66.3 | 9.8 | 28 | 80 |
| *E* Diesel fuel + 2½% 2, 4, 5-T | 63.7 | 9.9 | 20 | 57 |
| *F* Fuel oil + 2½% 2, 4, 5-T | 64.9 | 8.5 | Nil | — |
| *G* Diesoline + 5% 2, 4, 5-T | 64.4 | 10.1 | 33 | 94 |
| *H* Diesel fuel + 5% 2, 4, 5-T | 60.6 | 9.7 | 29 | 83 |
| *J* Fuel oil + 5% 2, 4, 5-T | 67.4 | 8.7 | Nil | — |
| *K* Diesoline + 2% 2, 4, 5-T + u/t | 68.2 | 9.2 | 17 | 49 |
| *L* Diesoline + 5% 2, 4, 5-T | 66.1 | 9.9. | 30 | 86 |
| *M* Control u/t | 64.2 | 8.6 | Nil | — |
| *O* Control d/t | 69.8 | 8.2 | Nil | — |

u/t = upward tapping

d/t = downward tapping

* = measurements taken one month after application of treatments

data for the control trees show that the thickness of the renewed bark was well above average for bark of second renewal of unselected seedling trees.

Observations on the effect of the poison on the foliage and the bark of the trees were commenced immediately after the application of the treatments on 18 July 1955. At first they were made weekly, later at fortnightly intervals up to April 1956, noting for each tree: when leaf discolouration commenced; leaf shedding started; all leaves had been shed; refoliation was observed; new leaves had been shed; treated bark was dead; latex flow had ceased; borers invaded the stem. A final round of inspection was made one year after the start of the experiment in July, 1958. As criterion for 'death' of the trunk, the following observations were combined: all leaves were shed; treated bark was dead; untreated bark would yield no latex; borers were in the trunk. When these conditions were met the trees were possibly not completely dead, but were at any rate dying without chance of recovery. The crowns were usually disintegrating before the bark completely ceased to yield latex, but when the bark was removed the cambium was found to be discoloured and black streaks were seen in the sapwood.

## RESULTS

### *Kill of Trees*

Table I lists the mean girth and bark thickness of the 35 trees allocated to each treatment, together with the number of trees killed one year after treatments were applied. The slight variation in girth between treatments is caused by the fact that the trees were allocated to the various treatments primarily on a basis of yield performance, girth being a secondary consideration.

Measurements of bark thickness were taken one month after the treatments were applied. The effect of 2, 4, 5-T on bark thickness can be seen on trees of treatments *D, E, G, H, K* and *L*. The fuel oil treatments with 2, 4, 5-T (treatments *F* and *J*) had no effect on bark thickness.

The following treatments have proved ineffective as tree poisons:

*A* Diesoline alone

*B* Diesel fuel alone

*C* Fuel oil alone

*F* Fuel oil + 2½% 2, 4, 5-T

*J* Fuel oil + 5% 2, 4, 5-T

From these observations it appears that diesel oils are not effective tree poisons when applied over a 15-inch full circumference band to the bark at the base of rubber trees. Furthermore it is shown that fuel oil is an unsuitable carrier for 2, 4, 5-T, because of its high viscosity and poor penetration.

Six treatments, effective in varying degrees, remain to be considered—those containing 2½% or 5% of 2, 4, 5-T in either Diesoline or diesel fuel. The results obtained with these materials have been summarised in Tables 2 and 3, Table 2 giving the effects of formulations containing 2½% 2, 4, 5-T and Table 3 those of formulations containing 5% 2, 4, 5-T.

TABLE 2. EFFECT OF 2½% 2, 4, 5-T FORMULATIONS

| Month after treatment | No. of trees dead | Mean girth |
|---|---|---|
| 1 | 14 | 44.9 |
| 2 | 9 | 56.0 |
| 3 | 20 | 62.7 |
| 4 | 6 | 73.9 |
| 5 | 10 | 70.1 |
| 6–11 | 14 | 67.6 |
| Number of survivors | 31 | 77.2 |
| Total number of trees treated | 104 | 66.1 |

TABLE 3. EFFECT OF 5% 2, 4, 5-T FORMULATIONS

| Month after treatment | No. of trees dead | Mean girth |
|---|---|---|
| 1 | 14 | 46.9 |
| 2 | 18 | 66.4 |
| 3 | 15 | 60.0 |
| 4 | 13 | 72.9 |
| 5 | 13 | 65.4 |
| 6–11 | 19 | 75.7 |
| Number of survivors | 13 | 81.2 |
| Total number of trees treated | 105 | 67.1 |

A breakdown of the figures by individual treatments, indicating the number of killed trees and survivors, is given in Table 4.

The following conclusions are drawn from the data presented in Tables 2, 3 and 4:

a None of the treatments has resulted in a 100% kill one year after poisoning. However, most of the 'survivors' show advanced symptoms of poisoning and are already partially dead. On such trees it is often observed that a narrow strip of bark winding spirally up the stem remains alive, and the branch it connects up with also remains alive, whereas other branches are already disintegrating.

b The most rapid and effective tree poison used in this experiment is the 5% concentration of 2, 4, 5-T in the thin fluid Shell Diesoline.

c Upward tapping appears to delay the effect of the poison.

Table 4. Number of Dead Trees per Month (Cumulative)

| Months after poisoning | Diesoline +5% 2, 4, 5-T | Diesel fuel +5% 2, 4, 5-T | Diesoline +5% 2, 4, 5-T | Diesoline +2½% 2, 4, 5-T | Diesel fuel +2½% 2, 4, 5-T | Diesoline +2½% 2, 4, 5-T |
|---|---|---|---|---|---|---|
| | d/t | d/t | u/t | d/t | d/t | u/t |
| 1 | — | — | — | — | — | — |
| 2 | 10 | 3 | 1 | 6 | 8 | — |
| 3 | 18 | 9 | 5 | 11 | 11 | 1 |
| 4 | 20 | 13 | 14 | 19 | 20 | 4 |
| 5 | 25 | 18 | 17 | 21 | 22 | 6 |
| 6 | 27 | 21 | 25 | 28 | 24 | 7 |
| 7 | 29 | 25 | 27 | 28 | 25 | 10 |
| 8 | 30 | 25 | 28 | 28 | 25 | 10 |
| 12 | 32 | 29 | 30 | 28 | 28 | 17 |
| Number of survivors | 2 (both dying) | 6 (five dying) | 5 (four dying) | 7 (five dying) | 7 (five dying) | 17 (twelve dying) |
| % survivors | 6% | 17% | 14% | 20% | 20% | 50% |

Before the treatments were applied, all trees were being tapped on a half spiral cut in renewed bark. After application of the treatments on trees which were to be tapped in an upward direction, new V/2 cuts were opened in virgin bark just above the renewed bark on the opposite side. This has, no doubt, caused a partial ringing effect which may have interfered with the translocation of the 2, 4, 5-T. The discolouration of the leaves after the application of the treatments was observed earlier on the downward tapped than on the upward tapped, trees of comparable treatments (Table 5).

TABLE 5. ONSET OF LEAF DISCOLOURATION FOLLOWING POISONING (CUMULATIVE NUMBER OF TREES)

| Months | Diesoline +2½% 2, 4, 5-T | Diesoline +5% 2, 4, 5-T | Diesoline +2½% 2, 4, 5-T | Diesoline +5% 2, 4, 5-T |
|---|---|---|---|---|
| 1955 | d/t | d/t | u/t | u/t |
| July | 5 | 6 | 1 | 2 |
| August | 17 | 17 | 5 | 12 |
| September | 22 | 23 | 8 | 19 |
| October | 29 | 29 | 9 | 20 |
| November | 30 | 34 | 23 | 33 |
| December | 34 | 35 | 24 | 33 |
| 1956 | | | | |
| January | 34 | 35 | 24 | 34 |
| February | 35 | 35 | 24 | 34 |
| Number of trees showing no discolouration | Nil | Nil | 10 | 1 |

Observations made elsewhere also support the view that trees tapped on several cuts on both sides of the stem react less rapidly to the 2, 4, 5-T tree poison. However, it would appear from the figures in Table 4 that if the concentration of 2, 4, 5-T is at the 5% level the effect of upward tapping is only a delaying one, the ultimate kill being nearly similar to that of the trees treated in the same way but tapped downwards. With the 2½% 2, 4, 5-T concentration a definite reduction in the number of trees killed was caused by upward tapping.

The time of leaf discolouration appears to depend more on the tapping system than on the concentration of 2, 4, 5-T in the Diesoline.

Leaf discolouration and subsequent shedding are, however, also governed by environmental conditions. On one estate a solid block of 120 acres of old seedling trees was poisoned with 5% 2, 4, 5-T in diesel fuel. Practically all trees were completely defoliated within a fortnight and a record seemed to have been established. Poisoning was

done in a severe dry spell of weather which continued for three weeks after application of the poison. With the advent of the rains, however, a very high proportion of the trees refoliated and the subsequent kill was slow and unsatisfactory.

No such climatic extremes have been experienced during the period covered by our experiment, in which 16% of the trees treated with 5% 2, 4, 5-T in either Diesoline or diesel fuel refoliated after complete or partial defoliation. All but three of these died in later months.

*d* Most of the surviving trees are of above average girth, and the figures show a trend, the less vigorous grown trees being more rapidly killed (Tables 2 and 3).

We know from experience that thinning in a young tappable stand of rubber can be effectively done with a 2½% or 5% concentration of 2, 4, 5-T in Diesoline. In the tree poisoning experiment under review there have, however, been a few poorly grown old seedling trees which were less rapidly killed than other, more vigorously grown, trees.

Irrespective of tapping systems roughly 74% of the trees poisoned with 5% 2, 4, 5-T in Diesoline were killed within six months after poisoning, and 10% survivors were counted after one year. Of the surviving trees belonging to these treatments there was only one which appeared healthy, though even in this case some effects of treatment could be observed on the bark. All other survivors were partially dead.

*Yield*

The effect of the various treatments on yield is shown in *Figure 1A, B* and *C*. The following observations are noted:

*a* Diesoline and diesel fuel have both induced a significant increase in yield over a period of between two and three weeks after application (*Figure 1A*). This effect is not shown by the viscous fuel oil. It would appear that any action which wounds or partly kills the bark of a rubber tree results in an increased latex flow. Slashing, a mild fire, scraping of the bark, and in this case painting of a large strip of bark with a penetrating mineral oil, all result in a temporary yield increase. Treatment with a yield stimulant may be listed in the same group. All these 'actions' result in the formation of new protective tissues, and it is suggested that the formation of these new wound tissues (a form of growth) is closely related to the increased rubber regeneration.

*b* Addition of 2½% or 5% 2, 4, 5-T to these oils has resulted in high, though transitory, yield increases (*Figure 1A*). One month after poisoning the yield had come back to control level; it then dropped steeply by subsequent defoliation and kill of the trees.

Fuel oil + 2½% 2, 4, 5-T had a slight effect on leaf colour but did not kill a single tree. The yield was increased over a period of two and a half months after which it dropped below control level. There is only a slight difference between diesel fuel and Diesoline as a carrier for 2½% or 5% 2, 4, 5-T with regard to yield (*Figure 1A* and *B*).

*c* Upward tapping has clearly resulted in a longer period of increased yield (*Figure 1C*). Profitable tapping can then be done for a period of two and a half to three months after poisoning. This is, of course, linked with the delaying effect of upward tapping (mentioned earlier) on the defoliation and kill of trees.

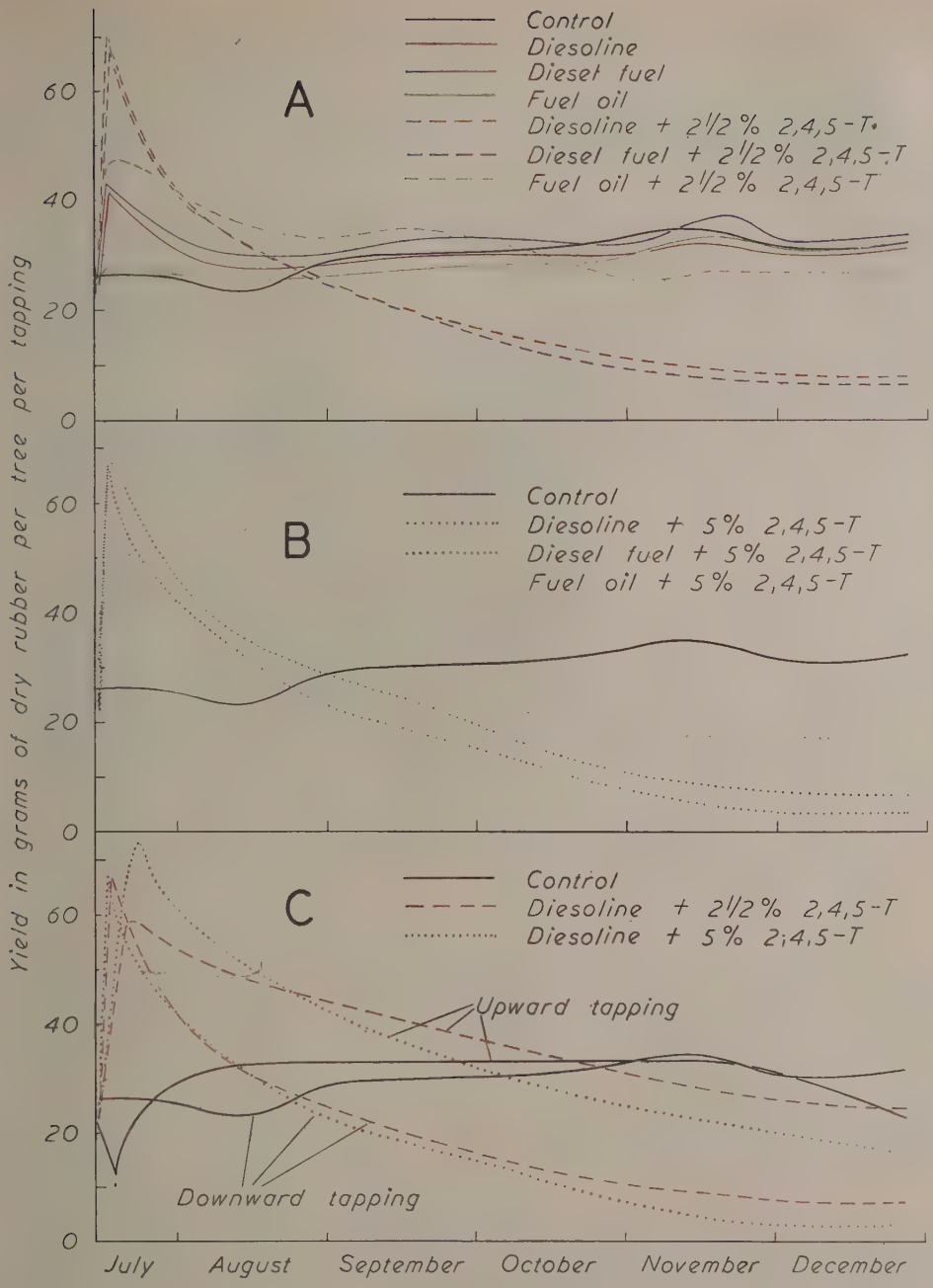


Figure 1. Comparison between treatment

When tapping in an upward direction the longest period of increased yield was noted for treatment Diesoline + 2½% 2, 4, 5-T which also resulted in a high number of survivors.

## CONCLUSION

Application of 2, 4, 5-T formulations as tree poisons have resulted in greatly increased yields of rubber trees.

The period of increased yield which lasts approximately one month on downward S/2.d/2.100% tapping can be lengthened to two and a half to three months when tapping V/2.d/2.100% in an upward direction in virgin bark.

No intensive tapping systems have been tried out in conjunction with tree poisoning, but our observations suggest that intensive multiple-cut tapping systems may interfere with the efficacy of the tree poison.

*Effect on the Root System*

A major object of poisoning old rubber trees is to obtain a rapid kill of the root system, which otherwise might become a serious source of root disease infection to the young replanting. When the root systems of a few poisoned trees in this experiment were exposed it was found that the lateral roots had remained alive for a much longer period than the trunk and branches. It was then decided to expose the root systems of all trees poisoned with a 5% concentration of 2, 4, 5-T in either Diesoline or diesel fuel, and also of the untreated control trees. The main lateral roots were exposed to a distance of four feet from the trunk and the taproot was exposed to a depth of four feet.

The effect of poisoning on the kill of roots one year after application of 2, 4, 5-T is summarised in Table 6.

Table 6. Kill of Roots One Year After Poisoning

| | Treatment | No. of trees | No. of laterals | Laterals % killed | Taproots % killed |
|---|---|---|---|---|---|
| G | 5% 2, 4, 5-T in Diesoline | 35 | 233 | 57% | 83% |
| H | 5% 2, 4, 5-T in diesel fuel | 35 | 254 | 54% | 86% |
| L | 5% 2, 4, 5-T in Diesoline (upward tapping) | 35 | 248 | 65% | 91% |
| O | Control | 35 | 255 | Nil | Nil |

It is shown that under the conditions of this experiment only 55—60% of the lateral roots were completely killed one year after poisoning with a 5% 2, 4, 5-T solution. However, many of the 'living' roots were partially dead and were already invaded with saprophytic fungi.

The observed slow kill of roots is not in accord with a statement in our publication 'Poisoning with 2, 4, 5-T' in *Planters' Bulletin 17*, page 41, the last paragraph of which reads 'Excavation some six months after poisoning has shown that all roots are killed by this time, as for sodium arsenite treatment'. At that time examination of the root system was mainly limited to the taproots and the bases of the laterals. It can be seen from Table 6 that nearly all taproots were dead and the junctions of the laterals with trunk or taproot were also nearly invariably dead. This has, unfortunately, led to an erroneous conclusion. When the root systems of the trees were exposed it was found that a large number of lateral roots were invaded by fungi. At our request an officer of the Pathological Division has made an inspection of all laterals and taproots, the results of which are summarised in Table 7.

Table 7. Fungi in Roots of Poisoned Trees

| Treatment | No. of trees | No. of laterals | % of laterals invaded by | | % of dead laterals not invaded |
|---|---|---|---|---|---|
| | | | *Ustulina zonata* | Other fungi | |
| *G* | 35 | 233 | 12.7% | 50.5% | 8.6% |
| *H* | 35 | 254 | 12.6% | 42.5% | 21.3% |
| *L* | 35 | 248 | 14.5% | 54.4% | 9.3% |
| *O* (control) | 35 | 255 | Nil | Nil | Nil |

The frequent occurrence of *Ustulina*, which is sometimes parasitic, on lateral roots of the poisoned trees should not be taken as evidence that the roots invaded by it were dead before the poison was applied to the trees. The lateral roots of the control trees did not have any *Ustulina* infection at all, showing that *Ustulina* has invaded the poisoned trees as a saprophyte.

The other identified fungi were *Polyporus rugulosis*, *Ganoderma applanatum*, and species of *Poria* and *Xylaria*. An unidentified brown fungus was also observed. These saprophytic fungi have been grouped together in Table 7 under the heading 'other fungi. *Ustulina* is listed separately because of its alternate parasitic habit, but the distinction is believed to be without significance.

It was further found that a proportion of the poisoned trees belonging to treatments *G*, *H* and *L* was invaded by termites. Twentynine out of the total of 105 poisoned trees were so affected and of these 16 were invaded by *Coptotermes curvignathus*. Five more trees had carton-like nests similar to that of *Coptotermes curvignathus* and the eight remaining trees were invaded by other species, some which have not been identified. None of the control trees was infested.

These pathological observations are put on record without further comment as experiments are in progress in which the effects of several tree poisoning methods are being compared, and from these more conclusive information is expected.

SUMMARY

The efficacy, as tree poisons, of a 2½% and 5% acid-equivalent concentration of the normal butyl ester of 2, 4, 5-trichlorophenoxy acetic acid in three different grades of diesel oil has been investigated in an experiment carried out on the R.R.I. Experiment Station. Seventy per cent of the trunks and branches of the treated trees were dead within six months, and practically all trees were dead or nearly dead one year after application of 5% 2, 4, 5-T in Shell Diesoline.

The killing effect on the lateral roots was much slower. Sixty per cent of the lateral roots were dead 12 months after poisoning. A record is given of pathological observations.

The yield of trees treated with a 2, 4, 5-T tree poison was greatly increased for a period of one to three months depending on the tapping system employed. It is suggested that full circumference tapping may interfere with the translocation of 2, 4, 5-T and may result in slower kill.

*The cooperation of the staff of the Pathological Division in identifying fungi and termites is gratefully acknowledged.*

*It is a pleasure to put on record the able field work of Mr R. Perumal and the accurate compilation of data by Mr Chen Khyun Thai.*

*Botanical Division*
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*Accepted February*

# *Natural Rubber Latex as Initiator for Vinyl Polymerisation*

B. C. SEKHAR

*A process is described by which it is possible to use ammoniated hevea latex as initiator for the polymerisation of vinyl monomers.*

VARIOUS METHODS HAVE BEEN PROPOSED in the literature for the polymerisation of vinyl monomers in latex. All these methods involve the addition of an initiator system consisting of a peroxide or hydroperoxide and an activator which is usually a reducing system or a polyamine [1-4].

Natural rubber latex has been found to contain an 'amine' which, in the presence of hydroperoxide, will cause polymerisation of vinyl monomers. Thus, a part of the initiator system is already available in natural rubber latex.

*Experiment 1*

Latex from two sources, one from the R.R.I. Experiment Station and the other from the R.R.I. holding in Ulu Klang, were used for this experiment. Ten ml of methyl-methacrylate containing 0.07 ml of cumene-hydroperoxide was mixed with 30 ml ammoniated field latex containing 0.5% on rubber of ammonium oleate. The maximum temperature attained in one hour and the dry yield on overnight standing were determined. Table 1 shows the efficiency of this naturally present 'amine' in latex, in causing polymerisation of methyl-methacrylate containing added cumene-hydroperoxide.

TABLE 1. POLYMERISATION EFFICIENCY OF LATEX 'AMINES'

| Sample | Temperature rise °C | Monomer polymerised % |
|---|---|---|
| Experiment Station bulk | 6 | 85 |
| Ulu Klang bulk | 10 | 90 |

It has further been shown that peroxidation takes place in ammoniated latex on standing or agitation, in the presence of oxygen. At ambient temperatures such peroxidation resulted mostly in the production of active centres on the polyisoprene. These active centres had hydroperoxide properties and were capable of polymerising vinyl monomers in presence of a reducing system.

*Experiment 2*

To 50 ml of activated latex, 15 ml of methyl-methacrylate was added followed by 0.15 ml of 1% ferrous sulphate and 1.5 ml of tetra-ethylene-pentamine. The maximum temperature attained in one hour and the dry yield on overnight standing were then determined. Table 2 shows the efficiency of peroxidised latex in causing the polymerisation of methyl methacrylate in the presence of ferrous sulphate and tetra-ethylene-pentamine.

COMMUNICATION 318

Table 2. Polymerisation Efficiency of Peroxidised Latex

| Sample | Temperature rise °C | Monomer polymerised % |
|---|---|---|
| Ordinary latex | 0 | nil |
| Activated latex (agitated in air for 96 hrs) | 20 | 88% |

It is thus possible to obtain the entire initiator system for polymerisation of vinyl monomers from natural rubber latex itself by combining the effect demonstrated in the first experiment with that of the second. This is clearly shown in the following experiment.

*Experiment 3*

A sample of agitated latex (25 ml) is mixed with ordinary field latex (25 ml) in the ratio of 1:1 and monomer (15 ml) containing 0.15 ml of ferrous-sulphate is added to it and the mixture left standing overnight. The resulting temperature rise and yield are given in Table 3. Polymerisation of vinyl monomers can thus be initiated by specially prepared natural rubber latices.

Table 3. Polymerisation Efficiency of Mixtures of Peroxidised Latex and Fresh Latex

| Samples | Temperature rise °C | Monomer poymerised % |
|---|---|---|
| Agitated latex + field latex | 15 | 80 |
| Agitated latex | 2 | 5 |
| Field latex | 0 | 0 |

It is well known that latices from different sources and clones vary considerably in their non-rubber content. This is particularly true in the case of the naturally present 'amine' in latex. The efficiency of polymerisation will therefore vary from latex to latex. This is clearly demonstrated in Table 4. Latices from different clones were mixed with methyl methacrylate containing cumene-hydroperoxide, as in the first experiment and the increase in temperature and yield on overnight standing measured.

Table 4. Polymerisation Efficiency of 'Amines' in Clonal Latex

| Sample | Latex | Temperature rise °C | Monomer polymerised % |
|---|---|---|---|
| 1 | Pil B84 | 10 | 100 |
| 2 | Pil D65 | 11 | 100 |
| 3 | AVROS 50 | 6 | 86 |
| 4 | PB 23 | 9 | 97 |
| 5 | BD 5 | 13 | 92 |
| 6 | AVROS 49 | 11 | 100 |
| 7 | Tjir 1 | 12 | 96 |
| 8 | PB 86 | 13 | 98 |

In addition to this variability of the 'reduction activator' present in latex, the capacity of latices to undergo peroxidation in the presence of air is itself also variable. In other words, latices stored in presence of air will peroxidise to different extents. This is clearly shown in Table 5. Latex concentrates from different clones were stored for one month in presence of air. These latices were then mixed with methyl-methacrylate and tetra-ethylene-pentamine and the increase in temperature and yield on overnight standing measured as in Experiment 2.

Table 5. Polymerisation Efficiency of Peroxidised Clonal Latex Concentrates

| Sample | Latex | Temperature rise °C | Monomer polymerised % |
|---|---|---|---|
| 1 | Pil B84 | 1.5 | 5 |
| 2 | Pil D65 | 6.5 | 33 |
| 3 | AVROS 50 | 4.5 | 26 |
| 4 | PB 23 | 4.7 | 24 |
| 5 | BD 5 | 7.6 | 35 |
| 6 | AVROS 49 | 8.5 | 43 |
| 7 | Tjir 1 | 0 | 0 |
| 8 | PB 86 | 8 | 40 |

The implications of these observations on graft polymerisation and a detailed study of the reactions involved will be published elsewhere. However it is evident from the work reported in this paper that these observations can form a basis by which the active 'amine' contents and extent of peroxidation of latex can both be measured. Such measurements are important for the latex industry since amines influence vulcanisation behaviour and peroxidation leads to changes in the physico-chemical properties of the rubber hydrocarbon.

*The author wishes to thank Mr W. G. Wren, the Head of Chemical Division, who initiated work on aeration of latex in these laboratories, for his valuable advice and suggestions. Thanks are also due to Mr Ho Kam Leng and Mr M. Balasundram for experimental assistance.*

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*June*

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# *Control of Coptotermes curvignathus Holmgren with Chlorinated Hydrocarbons*

A. NEWSAM and B. SRIPATHI RAO

*The nature of the attack on rubber trees by C. curvignathus is briefly described, with an indication of the degree of effectiveness of old-established control measures. Tests of a range of chlorinated hydrocarbon insecticides indicated that chlordane, aldrin and dieldrin are highly effective and give lasting protection against re-attack. The persistence of insecticidal activity in soil of chlordane, aldrin, dieldrin and endrin was assessed by exposing termites to treated soil in a standard manner. Six different soils were compared in this respect.*

COPTOTERMES CURVIGNATHUS HOLMGREN is the only species of termite known to attack living rubber trees in Malaya. Indigenous to the country, it persists in plantations where the remains of the original jungle have not completely wasted away, attacking trees of all ages. Though it can attack perfectly healthy trees, its entry is facilitated by root disease or other injury which destroys the latex barrier; newly planted stumps are particularly liable to be attacked, and are quickly killed, often without any termites appearing above the soil. Termites affecting a healthy tree search for an easy point of entry, covering their runways on the trunk with mudwork. A mature tree may show no outward sign of an attack and, though the tap root and trunk are hollowed out, the tree can be tapped normally until it falls or is blown over.

Being social insects living in concealment, termites are difficult subjects to study and, in consequence, not much is known about their behaviour. It is believed that the nest of *C. curvignathus* is subterranean built within a submerged log or tree stump, from which tunnels run in all directions, linking up secondary nests in hollow trees or timber. It is not feasible to locate the main nest in order to destroy it. Insecticidal treatment applied to attacked trees contacts relatively few workers and soldiers; though they may be destroyed, others will attack neighbouring trees or, after the insecticide ceases to be active, will re attack the same tree. Regular inspection of badly infested young rubber has been necessary to discover and treat affected trees before they are seriously damaged. The materials used for termite control for many years were: mercuric chloride applied by pouring a 1% solution round the tree (the 'liquid' method), calcium cyanide and sodium silicofluoride applied by opening up the collar of the tree and dusting the roots (the 'crater' method), and white arsenic applied by injecting a small amount of the powder into the earth runs on the trunk (the 'trail injection' method). NEWSAM (1953) compared the three methods in mature rubber and concluded that all three were effective in driving termites away from attacked trees, but that the incidence of attack was little affected. Trail injection with white arsenic was satisfactory on mature trees, but not on young trees because of its tendency to burn the bark; for the latter, the solution of mercuric chloride was preferred.

The outstanding success of chlorinated hydrocarbon insecticides, starting with the war-time discovery of insecticidal activity of DDT and gamma benzene hexachloride, naturally led to their application to termite control problems. An up-to-date review of literature on this subject is given by SCHMITZ (1956).

COMMUNICATION 319

### FIELD EXPERIMENTS WITH CHLORINATED HYDROCARBONS

Field tests with chlorinated hydrocarbons as soil insecticides against termites have been carried out on the Experiment Station of the Rubber Research Institute of Malaya since 1950, when an application of four ounces of benzene hexachloride dust containing 0.4% gamma isomer round the collar of mature trees was found to have no protective value. In the same year an unsuccessful attempt was made to protect stumps planted in an area heavily infested with termites. Either two ounces of benzene hexachloride dust containting 0.4% gamma isomer, or the same weight of 5% DDT dust or 5% toxaphene dust, was incorporated into the soil of the planting hole. The treatments did not prevent heavy losses of stumps.

A greater range of insecticides and formulations was available in 1952, some only as samples for experimental purposes. A test was put down in November of that year in a seven-acre low lying area planted with stumps. Dusts containing 0.5% gamma benzene hexachloride, 5% DDT or 5% toxaphene were incorporated into the soil of the planting hole at the rate of one pound per point. Wettable powders containing 0.065% gamma benzene hexachloride, 0.25% DDT or 0.25% toxaphene were watered round the stumps while planting, at the rate of two gallons per point, as were aqueous emulsions containing 0.025% gamma benzene hexachloride, 0.25% DDT, 0.187% chlordane, 0.12% aldrin or 0.09% dieldrin. The costs of the chemicals were taken into account in deciding the dosages applied.

The area was divided into plots of 100 contiguous points, each planted with two stumps. Each plot received one treatment, applied to 50 points only, alternating with untreated controls. In addition, three more treatments were combined with the three dust treatments. Wettable powders containing 6.5% gamma benzene hexachloride, 25% DDT or 25% toxaphene were suspended in a 2.5% tapioca flour paste at the rate of 2.5 lb per gallon; into these pastes the roots of additional stumps were dipped just before planting — as a third stump at each control point, about a foot and a half away from the untreated plants of the appropriate dust treatment.

Heavy incidence was expected as *C. curvignathus* was known to be present in some large jungle stumps in the area. Surprisingly, the number of points attacked during the first year was only twenty; thereafter there was no incidence in the treated area. Of the attacked points eleven were untreated controls compared with nine from the treated plants. These nine were made up of two treated with DDT dust, three with DDT paste, two with toxaphene dust and two with toxaphene paste. Compared with this, in the 45 acres of the rest of the field planted with stumps at the same time, and thought to be less liable to attack, the rate of incidence was two and a half times as high during the first two years.

Though the experiment did not bring out the relative merits of the different treatments, it indicated that benzene hexachloride, chlordane, aldrin and dieldrin might hold promise. It also indicated that emulsions are to be preferred to dusts, wettable powders and pastes.

Aqueous emulsions of these four insecticides were further tested for their protective value for stumps planted in November 1953, in a four-acre block. There were five plots each containing approximately 170 planting points with one stump; a central plot was left untreated while the surrounding four were treated with gamma benzene hexachloride, chlordane, aldrin and dieldrin at the same concentrations as in the previous experiment, but at the rate of half a gallon per point instead of two gallons. In the treated plots, too, one untreated control plant was left after every two treated points. Half the quantity of the insecticide was poured into the planting hole and the other half round the stump after filling the hole with earth.

Only fifteen plants were attacked during the first four months and none for eighteen months after that. The fifteen attacked plants comprised three treated with benzene hexachloride, five controls in the same plot, four controls in the chlordane treated plot and three controls in the dieldrin treated plot. There was a complete absence of attack in the central control plot during this period.

The experiment, apart from suggesting that benzene hexachloride is inferior to chlordane, aldrin and dieldrin, strengthened the belief that the latter are effective at low dosages and that their widespread use in an area may affect the whole colony, resulting in a drastic reduction in incidence.

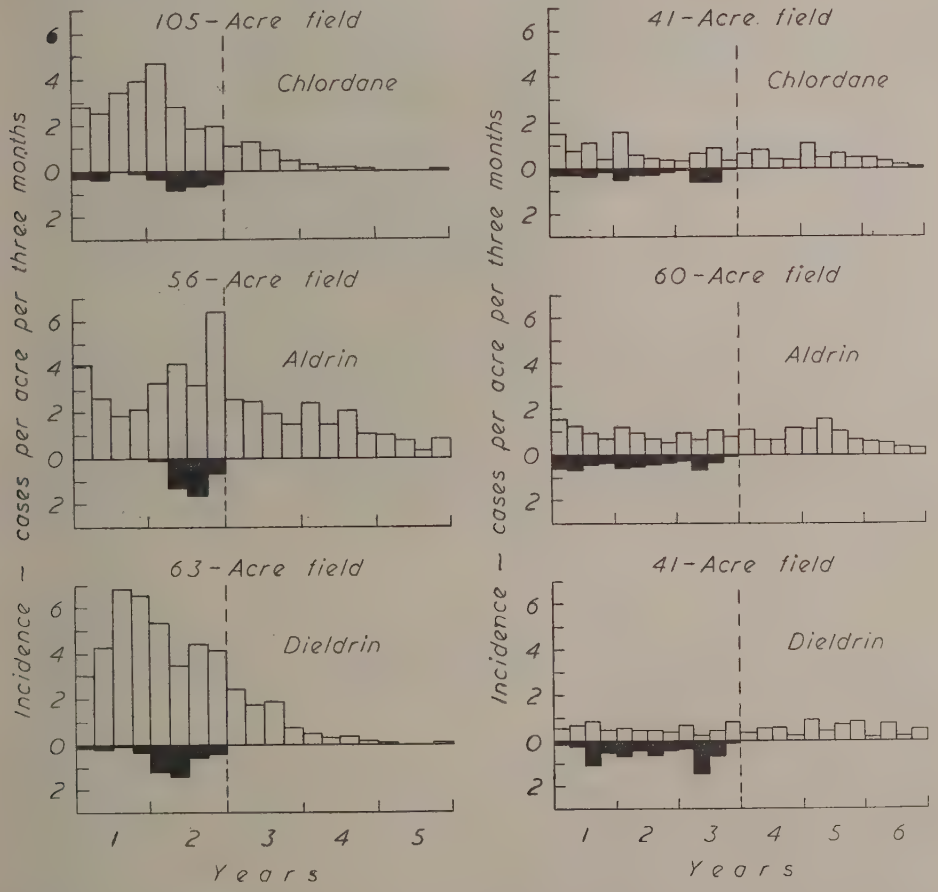


*Figure 1. Termite incidence in three fields of immature (left) and mature rubber (right) under different treatments.*

The low rates of attack in experimentally treated areas led to a different approach being tried to compare the relative effectiveness of these materials. Over the whole of a field of immature rubber one experimental treatment was given to attacked plants discovered during fortnightly inspections, the incidence rate being compared with that previously found under a standard treatment (one pint of 1% mercuric chloride). The four materials were tested at the rate of one pint per tree of the same concentrations as used before — 0.025% gamma benzene hexachloride, 0.187% chlordane, 0.12% aldrin and 0.09% dieldrin.

A few cases of re-attack of treated trees were noticed, some even within a month after treatment, in the field receiving the benzene hexachloride treatment, which was accordingly discontinued after six months. In the fields treated with chlordane, aldrin and dieldrin, the rate of incidence declined and treated trees remained free of reinfestation for over three years, except for three trees out of 980 treated with aldrin and one out of 532 treated with dieldrin. *Figure 1* illustrates the incidence of attack (clear) and re-attack (shaded) per acre per three months, in three fields, for two years under treatment with mercuric chloride and for three years under treatment with the new insecticides.

The minimum effective dose of these insecticides was still to be found; for this purpose they were used at the following reduced concentrations: chlordane 0.075% and 0.0375%, aldrin 0.05% and 0.025% dieldrin 0.03% and 0.015%, applying one pint of the emulsion per attacked tree. Over a period of more than two years there have been only few cases of re-attack of treated trees, and these have occurred where the soil is peaty. The effect of peat will be discussed later.

Following this success, chlordane, aldrin and dieldrin replaced mercuric chloride and white arsenic for routine treatment on the Experiment Station. In fields of immature rubber the diluted insecticides were used at the rate of one pint per tree, but in mature rubber the rate of application was doubled to allow for greater girth of the trees. In contrast with fields of immature rubber, the decline in incidence in mature fields was slow, though there were no cases of re-attack. *Figure 1* also illustrates the incidence of attack (clear) and re-attack (shaded) per acre per three months in three fields of mature rubber, for three years under the old treatment and three under the new. The slower decline may be explained by the large trees harbouring the pests for many years before they could be discovered and treated.

## SOIL TESTS

The relative effectiveness of these insecticides, and the time they will remain active in the field, was studied by exposing termites to samples of treated soil in a standard manner. Tests of this nature have been described by Hockenyos (1939), Shelford (1949) and Hetrick (1950).

*C. curvignathus* workers will survive for many days in petri dishes provided with moist filter paper for food and to keep the humidity high. For the test, the soil sample was evenly spread over the filter paper in a 9 cm dish, into which were placed ten workers selected from large numbers collected two days prior to the test. High humidity was maintained by spraying water on to the soil with a hand atomizer whenever the inner surface of the lid was found to be free of condensation.

The tests were made with samples of soil from fields that had received experimental treatments described earlier. The soil generally in these fields is a fine sandy loam with patches of peat overlying alluvial sand. Peaty areas were avoided when taking soil samples; otherwise trees were taken at random and soil collected from about two inches below the surface and close to the collar. Tests were repeated at intervals of

three months, each time selecting a different tree for sampling. Duplicate dishes were set up with each soil sample, and there was an extra pair of dishes with untreated soil, as a check on the viability of the termites used in the test. Daily counts of dead and dying workers were made for two weeks. It was found that, in treated soils which retained their toxicity, the termites were in a dying condition at the end of twentyfour hours — lying on their backs with their legs twitching — and were dead by the second or third day. In soil which was only feebly toxic the termites, though affected, were still mobile after the first day, and they would take nearly a week to die.

SURVIVAL OF TERMITES PLACED IN TREATED SOIL

| Concentration of insecticide applied | Period since soil treated (months) | Condition of termites after 24 hours exposure | Time for death (days) | Assessment of soil toxicity |
|---|---|---|---|---|
| 0.0375% chlordane | 15 | dying | 4 | toxic |
| | 18 | affected | 6 | feebly toxic |
| | 21 | affected | 7 | feebly toxic |
| | 24 | active | 10* | non-toxic |
| 0.025% aldrin | 15 | dying | 2 | toxic |
| | 18 | affected | 5 | feebly toxic |
| | 21 | affected | 5 | feebly toxic |
| | 24 | active | 10* | non-toxic |
| 0.015% dieldrin | 27 | dying | 2 | toxic |
| | 30 | affected | 5 | feebly toxic |
| | 33 | affected | 6 | feebly toxic |
| | 36 | active | >14 | non-toxic |
| 0.187% chlordane | 36 | dying | 3 | toxic |
| 0.12% aldrin | 36 | dying | 3 | toxic |
| 0.09% dieldrin | 36 | dying | 3 | toxic |
| 0.025% gamma BHC | 3 | active | >14 | non-toxic |
| 1% mercuric chloride | 1 | active | >14 | non-toxic |

* death due to fungus attack.

In preparing the Table, where the more significant results are set out, the early observations repeatedly showing the soils to be still toxic have been omitted, leaving only those which show when toxicity was being lost. In all the treatments recorded in the Table, the quantity of diluted insecticide applied round each tree was one pint. It is seen that 0.0375% chlordane and 0.025% aldrin persisted equally well, and that the concentration of dieldrin to give a comparable result would be less than 0.015%.

Further, the excellent retention of the higher concentrations of the same three materials, which remained fully toxic after three years, contrasts strikingly with the results obtained with gamma benzene hexachloride and mercuric chloride.

The poor performance in peat, already referred to, suggested that persistence might depend on soil type. To investigate this a pot test was carried out, starting in December 1954, with six soils: fine sandy loam, coarse sandy loam, lateritic soil, volcanic soil, coastal alluvial clay, and peaty soil. Emulsions of chlordane, aldrin and dieldrin, and of endrin (here tested for the first time) were all diluted with water to the same concentration of the active ingredient (0.05%) for mixing at the rate of 500 cc to six pounds of air-dry soil, in an eight-inch pot. The pots took only half the quantity of peat. The four sets of six pots, and a set of six pots containing untreated soils, were buried in an open place to about one inch from the rim. Each set was widely separated from the others, to prevent contamination.

At monthly intervals the top three inches of soil was mixed and a sample taken into the test petri dish as before. The method of testing was made more sensitive than in the previous experiment, twenty termite workers being introduced into the dish for two hours only, after which they were transferred ten each to two petri dishes prepared with a small quantity of untreated soil of the same type. Observations on their condition were made immediately after exposure to the treated soil, again after four hours, twentyfour hours, and thereafter daily. While a treated soil retained its full toxicity, termites exposed to the sample appear feeble and are slow in their movements either at the time of transfer to untreated soil or within four hours after that, and are in a dying condition after twentyfour hours. As the toxicity diminished, the symptoms appeared more slowly. Termites which were still active three days after exposure generally lived for many days more. This criterion was taken as indicating that the soil had lost its toxicity. The periods taken for the different insecticides to be lost in the various soils are represented in *Figure 2*. After the forty months that the experiment has been running, dieldrin has lost its toxicity in clay but remains active in all the other soils with the exception of peat.

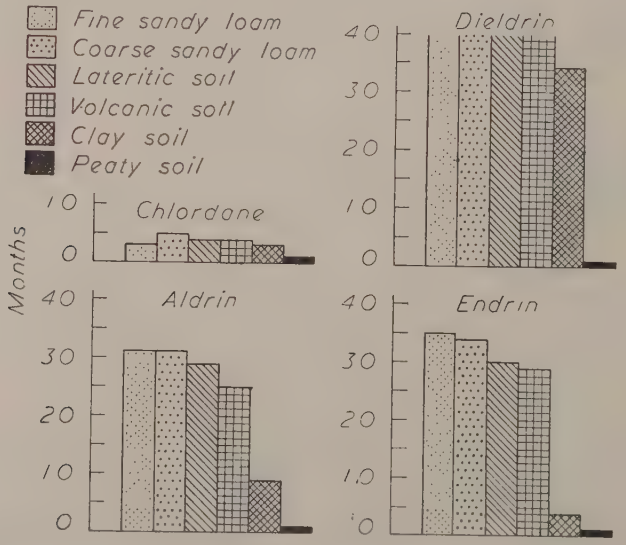


*Figure 2. Persistence of chlordane, aldrin, dieldrin and endrin in different soil types.*

## DISCUSSION AND CONCLUSIONS

The insecticide chlordane and those related to it—aldrin, dieldrin and endrin — can be said to have solved the long existing problem of termite control in rubber plantations. In addition to being effective in soil at extremely low concentrations, their activity persists for long periods, forming an effective barrier against future attack. Applied to the soil in equal amounts chlordane becomes ineffective much earlier than the other three insecticides; dieldrin remains effective longest. Endrin, which persists about as well as aldrin, is excluded from practical use by its relatively higher price. To be about comparably persistent, the relative concentrations of dieldrin, aldrin and chlordane need to be approximately in the ratio 1:2:3.

Tests with different soil types have shown all the insecticides to be lost sooner in a coastal alluvial clay than in four inland soils tested. In peat they are all quickly inactivated, but it has been found, using aldrin, that the effect can to some extent be overcome by a large increase in dosage.

The cost of these insecticides at the very low rates of application needed is so small that it is feasible to treat every tree in an infested area, which will be effectively protected for a few years, the time depending on the soil type and the dosage. Further, their low toxicity to mammals makes them much safer materials to use than mercuric chloride and white arsenic, which have been the standard treatments for many years.

*Apart from the inclusion of a further six months records in Figure 2, the paper is as presented to the Ninth Pacific Science Congress, Bangkok, 1957. It is published with the permission of the Organising Committee.*

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*June*

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# *Superior Processing Rubber Masterbatch*

B. C. SEKHAR and G. W. DRAKE

*Two processes are described for imparting superior processing characteristics to all grades of rubber. The first process, which makes use of a dry vulcanised rubber masterbatch, is best used in consuming areas to impart SP properties to RSS 2, 3, 4 and 5 grades. The second process, which makes use of the vulcanised rubber masterbatch coagulum, is best used in the producing areas to impart SP characteristics to the remilled grades.*

THE ADVANTAGES OF SUPERIOR PROCESSING RUBBERS[1] in extruding, calendering, reworking and pan curing have all been accepted by the rubber manufacturing industry. Three hundred tons were sold to the industry in 1957 and, with the publication of a Technical Bulletin[2], more usage is expected in 1958. The process[3] which involves the admixture of 20 parts vulcanised rubber with 80 parts of unvulcanised rubber in latex form has some marketing disadvantages, the main one being that by this process SP rubber can only be made in No. 1 quality grade and cannot be prepared in any other grade. Lower grades of smoked sheet are mainly produced by smallholders. It would be extremely difficult to incorporate vulcanised latex into smallholders' latex in the same way that present SP rubbers are made. The lower grades of crepe are made from various kinds of wet scrap rubber. Mixing wet scrap with vulcanised latex does not result in a rubber with SP properties. Many goods in which the use of SP rubber would prove advantageous are made from lower grade rubbers and the advantages in processing offered by SP rubber cannot outweigh the additional cost of the higher grade. Efforts at the Institute were therefore directed towards finding a method of conferring superior processing characteristics upon all grades of rubber. This paper describes two successful processes developed for such a purpose; these are known as the Dry Masterbatch and the Wet Masterbatch processes respectively. Patent application has been made to cover them and is pending.

### *Dry Masterbatch Process*

It is generally known that completely vulcanised rubber is incompatible with raw rubber on the mill. If it were not, the problem would have been simple. The reasons for the failure are crumbling of vulcanised rubber on the mill and the large differential between the raw Mooney viscosities of vulcanised and unvulcanised rubbers. In the standard SP rubber process, intimate blending of 80 parts of raw rubber with 20 parts of vulcanised rubber is achieved by mixing in the latex form. This SP rubber containing 20% of vulcanised rubber has been proved to be easily compatible with ordinary raw rubber on the mill. What is necessary then, is to determine the smallest proportion of unvulcanised rubber latex that has to be added to vulcanised latex to give a product that will knit well on the mill. If the vulcanised rubber proportion is sufficiently high in the product, then it should be possible to blend this material with any grade of rubber in appropriate quantities to impart SP characteristics to that grade of rubber. Investigations were therefore carried out by mixing different proportions of vulcanised and unvulcanised rubber latex, and studying the properties of the coagulated products obtained from such mixtures. The

COMMUNICATION 320

results obtained are summarised in Table 1 from which it is clear that as little as 10 parts of unvulcanised rubber mixed with 90 parts of vulcanised rubber in latex form imparts to the mixture sufficient tack to adhere together into a sheet on the open mill. The compatibility of these mixtures with further quantities of raw rubber on the open mill was then tested by blending the mixtures with appropriate quantities of raw rubber so that the final concentration of vulcanised rubber in the blend was in each case 20%. Typical results of tests of homogeneity, percentage extrusion swell, and scorch time, are given in Table 2.

TABLE 1. PROPERTIES OF DIFFERENT VULCANISED RUBBER MASTERBATCHES

| Vulc/unvulc rubber ratio | Appearance of coagulum | Appearance of crepe | Behaviour on the mill when dry |
|---|---|---|---|
| 98/2 | Fine crumb | Powders | Does not knit |
| 95/5 | Slightly coarse crumb | Very brittle | Knits but brittle |
| 90/10 | Coarse crumb | Brittle | Knits satisfactorily |
| 80/20 | Coarse crumb | Brittle | Good |
| 70/30 | Brittle coagulum | Slightly brittle | |
| 65/35 | Brittle coagulum | Satisfactory crepe | |
| 60/40 | Hard coagulum | Good crepe | |
| 50/50 | Normal coagulum | Good crepe | |
| 20/80 | Normal coagulum | Good crepe | |

TABLE 2. EFFECT OF MASTERBATCH CONCENTRATION ON PROPERTIES OF THE FINISHED SP RUBBER

| Vulc/unvulc rubber ratio | Wt of masterbatch (gm) | Wt of raw Yellow Circle rubber (gm) | Homogeneity of blend | % * Extrusion swell | Scorch † time in minutes |
|---|---|---|---|---|---|
| 98 : 2 | 51 | 199 | Poor | 17.9 | 9 |
| 95 : 5 | 52.5 | 197.5 | Slightly rough | 15.0 | 8 |
| 90 : 10 | 55.5 | 194.5 | Satisfactory | 14.8 | 8 |
| 80 : 20 | 62.5 | 187.5 | Good | 13.8 | 7 |
| 70 : 30 | 71.5 | 178.5 | Good | 14.3 | 8 |
| 65 : 35 | 77 | 173 | Good | 15.9 | 7.5 |
| 60 : 40 | 83 | 167 | Good | 14.2 | 8 |
| 50 : 50 | 100 | 150 | Good | 14.6 | 10 |
| 20 : 80 | 250 | — | Good | 19.3 | 8 |
| 0 : 100 | — | 250 | — | 30.2 | 9 |

* The % extrusion swell[1] is the % increase in cross-sectional area of a tyre tread compound of the test rubber after extrusion through an orifice of known dimensions under controlled conditions. The cross-sectional area of the extruded rod is calculated from weight and density measurements.

† The scorch time is measured by the tangent method[4] described by J. M. Buist.

By using a materbatch containing up to 90% vulcanised rubber and milling in the requisite amount of raw rubber to give a final concentration of 20% vulcanised rubber, the superior processing properties achieved in this experiment are, if anything, better than those obtained by conventional mixing in latex form. All grades of raw rubber can be used with such a masterbatch, as is shown in Table 3, in which the results obtained by blending the masterbatch with ADS, RSS and scrap crepe are shown.

Table 3. Properties of SP Rubbers Prepared using Dry Masterbatch

| 90 : 10 Vulc/unvulc rubber masterbatch (gm) | ADS | RSS | Bark scrap crepe | Homogeneity on the mill | % Extrusion swell | Scorch time (min) |
|---|---|---|---|---|---|---|
| 50 | 175 | — | — | Satisfactory | 19 | 9 |
| 50 | — | 175 | — | Satisfactory | 14.2 | 10 |
| 50 | — | — | 175 | Satisfactory | 19 | 7 |
| — | 225 | — | — | — | 32 | 9 |
| — | — | 225 | — | — | 30.2 | 9 |
| — | — | — | 225 | — | 37.1 | 11 |

There is, however, a considerable difference in Mooney viscosity between the masterbatch and the raw rubber with which it is mixed. Blending on an open mill is satisfactory provided that the masterbatch is added to the raw rubber before any serious breakdown has taken place.

*Recommended Procedure for the Dry Masterbatch Process*

Ordinary field latex is well mixed with vulcanised latex in the proportion of 1:9 based on rubber content. The mixture is coagulated in the usual manner, creped, the crumbled crepe is put on trays and dried at 120°F. The drying rate of the masterbatch crepe is fast and normally a ½ to 1 inch layer dries in 24 hours. The dry crumbs are then passed once through a dry creping mill to convert into sheet. The recommended packing procedure is to compress the sheets into bales, bind them with steel tapes, and use sisalkraft paper as an outer wrapper. Such bales have been sent to the United Kingdom and arrived at their destination in a satisfactory condition.

*Economics of the Dry Masterbatch Process*

The operating cost of the process can best be worked out as the extra cost involved over the cost of producing RSS 1. The following analysis is applicable for the production of 1000 lb of dry 90/10 SP masterbatch per day.

*1 Chemical for 1000 lb of masterbatch.*

| *Chemicals* | *lb* | *Price per lb ($)* | *Cost ($)* |
|---|---|---|---|
| Ammonia | 12 | 0.64 | 7.68 |
| Sulphur | 18 | 0.16 | 2.9 |
| Zinc oxide | 6.75 | 0.58 | 3.92 |
| Z.D.C. | 1.8 | 2.25 | 4.05 |
| M.B.T. | 3.6 | 1.27 | 4.57 |
| Dispersol LR | 0.003 | 1.6 | 0.01 |
| Extra formic acid for coagulation | 7.7 | 0.41 | 4.92 |
| | | *Total for chemicals* | 28.05 |

*2 Labour + electricity + steam for vulcanisation*—$8.84

*3 Drying and creping*—No additional operating costs for drying above the cost of making smoked sheets are envisaged. Indeed it is expected that there will be some saving in drying costs as the crumb dries faster than smoked sheets.

Since the dry crumb is passed once through the dry creping mill, there will be additional labour and operating costs which will be: $2.00

The production costs above RSS I thus amount to $38.89, which is 3.89 cents per lb.

*4 Depreciation of capital equipment*—In addition to the equipment required for vulcanisation, namely a boiler, a ball mill, a vulcanisation tank, bulking tank and stirrers, the following items are required:

Trays for holding the crumb,
modifications to existing drying sheds, and
one smooth roll creper.

Allowing ten year life, depreciation cost for all equipment above that used for RSS, approximates to 2.5 cents per lb.

*5 Packing charges*—The finished material occupies a 30% greater volume than normal sheet and therefore incurs additional freight charges which amount to about 1.2¢ per lb in shipment to the U.K. The total additional costs incurred in making 90/10 masterbatch thus amount to 7.59¢ per lb. A reasonable price including overheads and profits would be 11¢ over the price of RSS 1.

*6 Use of SP masterbatch*—Sufficient masterbatch is required to give 20% vulcanised rubber after mixing with raw rubber, that is, 2 parts of 90/10 masterbatch mixed with 7 parts of raw rubber. Assuming a differential of 3¢, 9¢ and 16¢ for RSS 2, RSS 3 and C Blanket respectively over the price of RSS 1, the increased cost of the final SP rubber in each case will be as follows:

$$\text{SP RSS 2} = \left(\frac{3}{9} + 11\right)2 = \frac{28}{9} = 3.1¢ \text{ over the price of RSS 2}$$

$$\text{SP RSS 3} = \left(\frac{9}{9} + 11\right)2 = \frac{40}{9} = 4.4¢ \text{ over the price of RSS 3}$$

$$\text{SP C Blanket} = \left(\frac{16}{9} + 11\right)2 = \frac{54}{9} = 6¢ \text{ over the price of C Blanket.}$$

*Wet Masterbatch Process*

An alternative method of imparting SP properties to lower grades has been developed by using masterbatch coagulum before drying. The use of this method is confined to producing countries, i.e. the plantation factory or the remilling factory. The principle of the method is similar to that of the dry process. The compatibility of the wet masterbatch with scrap used for preparing remilled crepe was first ascertained to determine the highest concentration of vulcanised rubber masterbatch that could advantageously be used. Masterbatches containing various proportions of vulcanised rubber were blended with scrap crepe on a creping mill. Typical results obtained are given in Table 4, which shows that the highest percentage of vulcanised rubber in the wet masterbatch that can successfully be incorporated into wet scrap is 80%. Trials on a large scale were then carried out at a remiller's factory, milling 90/10, 80/20, 70/30 and 50/50 wet masterbatches separately with scrap so that the final concentration of vulcanised rubber in each blend was approximately 20%. The resulting rubbers were dried and tested for extrusion swell and scorch time. Results obtained are given in Table 5.

Table 4. Compatibility of Wet Masterbatches with Scrap Rubber

| Masterbatch | Observations on compatibility with wet scrap |
|---|---|
| 95/5 | Very poor and difficult |
| 90/10 | Compatible, but will cut down production rate to half. Final sheets are too brittle to be hung for drying |
| 80/20 | Satisfactory in all respects — no cut in production rate |
| 70/30 | Very satisfactory all round |

Table 5. Properties of SP Lower Grades Prepared with Wet Masterbatch

| Wet masterbatch used vulc/unvulc ratio | Compatibility | % Extrusion swell | Scorch time (min) |
|---|---|---|---|
| 90/10 | Satisfactory but slow | 19 | 7 |
| 80/20 | Satisfactory | 18.7 | 7 |
| 70/30 | Good | 17 | 8 |
| 50/50 | Good | 18.5 | 8 |
| 20/80 | — | 19.3 | 8 |
| Control scrap used for blending | — | 37.1 | 11 |

*Recommended Wet Masterbatch Process*

The wet process therefore consists in first preparing a blend of 80 parts of vulcanised rubber and 20 parts of unvulcanised rubber in latex form. This master-

batch latex is coagulated in the usual manner, resulting in a crumb coagulum, which is mixed without drying with sufficient scrap on the creping battery to give a final rubber mixture containing 20% vulcanised rubber. The dry rubber content of the wet masterbatch can be accurately ascertained during its preparation, but the d.r.c. of the wet scrap can only be estimated by visual methods normally used by re-millers. However, the experiments and trials carried out indicated that the visual estimation of the d.r.c. of wet scrap was sufficiently accurate to impart more or less uniform SP properties to the final rubber.

*Economics of the Wet Masterbatch Process*

*Chemicals for 1000 lb of wet masterbatch of 80/20 vulc/unvulc composition*

| Chemicals | lb | Price/lb ($) | Cost ($) |
|---|---|---|---|
| Ammonia | 12 | 0.64 | 7.68 |
| Sulphur | 16 | 0.16 | 2.57 |
| Zinc Oxide | 6 | 0.58 | 3.49 |
| Z.D.C. | 1.6 | 2.25 | 3.6 |
| M.B.T. | 3.2 | 1.27 | 4.06 |
| Dispersol LR | .0027 | 1.6 | 0.01 |
| Extra formic acid | 6.84 | 0.41 | 4.92 |
| | | *Total for chemicals* = | 26.33 |
| | | *Labour, electricity and steam for vulcanisation* = | 8.33 |
| | | *Total extra manufacturing cost* = | 34.66 |
| | | *Total* = | 3.47¢ per lb. |

There are no other additional operating charges because the masterbatch is not dried but is mixed on the creping mill with wet scrap rubber and dried in the usual way.

*Depreciation of capital equipment*—: 1.5¢ (as with normal grades of SP rubber for extra equipment over RSS).

Thus, the total additional costs incurred in making 80/20 masterbatch amount to 4.97 cents per lb above RSS 1. A reasonable price including profit would be 7 cents per lb above the price of RSS 1.

*Use of Wet SP Masterbatch*

One part of 80/20 masterbatch is mixed with 3 parts of scrap rubber to produce 4 parts of SP brown crepe. Assuming a price differential of 16 cents between RSS 1 and scrap crepe, the additional cost of SP brown crepe must therefore be $\frac{7 + 16}{4} = 5.75$ cents over the equivalent scrap crepe. There is, however, an additional factor to be taken into consideration. Since there is approximately 1 part of good quality rubber mixed with 3 parts of scrap crepe, the final blended crepe is of improved colour over its equivalent grade of crepe. In other words, a part of the increased cost, say 0.75 cents/lb, can be compensated for by the improvement in price because of the better colour. This leaves 5 cents/lb as the final premium for imparting SP properties to the scrap grades by employing the wet masterbatch process.

## CONCLUSION

Two processes have been developed for imparting SP properties to low grades of rubber. The dry masterbatch process, which aims at selling a masterbatch as a compounding aid to manufacturers, can be blended with any grade of rubber, using normal mixing equipment. The wet process, however, is suitable only in producing areas. Although with the dry masterbatch any grade of rubber can be given SP properties the wet process can be used only to prepare SP remilled or scrap grade rubbers. The cost analyses that have been given in this paper favour the wet process for remilled grades. On the other hand the flexibility of the dry process and its utility with all grades of rubber makes it attractive. However, both processes are necessary to cover economically the entire range of rubber grades below RSS 1. The dry process should be used when SP RSS 2, 3, 4 and 5 are required, the wet process when SP remilled or scrap grades are required. Thus by a combination of the normal, dry and wet processes, it is now possible to produce SP rubbers in all grades.

At first sight the additional cost of SP lower grade rubbers may appear high. There are, however, several hidden advantages which cannot be estimated without actual experience of using SP lower grades in a factory. For example, an SP lower grade contains a proportion of first quality rubber and therefore has better physical properties than the normal material of that grade. The rate of mill breakdown is improved. In addition to this factor, the SP lower grades will contain appreciably less dirt and also will be of improved appearance and colour.

Although it is clear that the dry masterbatch can be also used for RSS 1, the advantage of obtaining the finished material without having to process it further to make it superior processing, by the manufacturers should be considered. In other words, where SP No. 1 grades are involved, it will be preferable to use the normal SP process. The two processes outlined in this paper are not therefore developed to compete with the existing process, but are a result of an attempt to meet the demands for lower grade superior processing rubbers.

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*Rubber Research Institute of Malaya*
*Kuala Lumpur* *June*

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# *Inoculation Experiment with Fomes lignosus, Klotzsch*

K. P. JOHN

*An experiment is described in which the roots of Hevea brasiliensis trees five or twentyfive years old were inoculated with white root disease. The inocula were pieces of root naturally infected with Fomes lignosus. By physically preventing external growth of the fungus on the roots, the rate of spread of infection was virtually halted. Incidental observations have been made on the penetration of F. lignosus and on host reaction.*

FOMES LIGNOSUS IS THE COMMONEST CAUSE of losses from root disease in *Hevea brasiliensis* during the first five years in the life of the plantation (SANDERSON 1930), (NAPPER 1932a). It can infect and kill trees of all ages. Infection of a healthy tree takes place when a root comes into contact with a diseased root in the soil. The rhizomorphs of the fungus spread inwards from the point of contact to the collar, then outwards along other lateral roots. Neighbouring trees are in turn infected and the process is repeated if control measures are not taken. The external growth of the fungus proceeds ahead of the region where the root is penetrated and killed (NAPPER 1932b). The rate of spread of the rhizomorph thus normally determines the rate of spread of the disease. It was not known to what extent the disease could spread internally in an infected root, and it is this that has been investigated, by physically suppressing the external growth.

## EXPERIMENTAL METHOD AND MATERIALS

The experiment to be described was carried out at the R.R.I. Experiment Station, Sungei Buloh, Selangor. The root systems of fourteen five-year-old trees and ten twentyfive-year-old trees were exposed to permit the selection of twenty lateral roots of each group. These roots were chosen as being reasonably straight, at a depth of not more than nine inches, and ranging in size from three quarters of an inch to two inches in diameter. Any branch roots or roots in contact with them were removed.

The young trees were growing in a well drained site on peaty sand that had been cleared from swamp in secondary jungle six years ago. Of the twenty roots inoculated in this area five were near a drain edge where the soil is a coarse sand with but little peat in it. A sparse growth of natural cover plants was present in the tree inter-rows but the selected roots were in the clean-weeded planting rows. The old trees were growing in a low lying site between two small ravines where the soil is a sandy loam, and five of their selected roots were also near a drain edge where the soil is a coarse sand with little organic matter in it.

All the roots were inoculated with *F.lignosus* by burying in contact with them sections of naturally infected wood, well penetrated, but still firm. The inocula, all obtained from the same source, varied in volume from 12 to 61 cubic inches. They were placed anywhere between 3 and 7½ feet away from the collar.

One month after inoculation an inspection hole was dug one foot away from the inoculum to expose about 7 inches of the proximal part of each root. For the duration

COMMUNICATION 321

of the experiment these holes were kept covered with roofing tiles with a layer of earth over them to prevent the desiccation of rhizomorphs growing along the root in the hole, and to maintain the temperature close to that of the surrounding soil.

All the inspection holes were opened at weekly intervals and, with a stiff brush, the mycelium was removed from the exposed section of one half of the roots of each age group. These roots are hereinafter referred to as the treated roots. The remaining ten roots of each age group were left untouched except for the weekly opening of the holes to note the progress of the disease and to give the same degree of exposure as was received by the treated roots. These roots are hereinafter referred to as the control roots.

## RESULTS

All the forty roots were dug out and examined a year after the start of the experiment.

In eight roots of the young trees and twelve roots of the old trees there was complete death either at the point of inoculation or for some further length on either side of it. Partial death at the point of inoculation was found on eight young roots and five of the old. In two roots each of the young and old trees there was only restricted penetration through wounds. There was no penetration at all in two of the young and one of the old roots, despite the fact that there had been vigorous superficial growth of the fungus over them in the early part of the experiment.

The results presented in Tables 1 and 2 show that preventing surface growth on the treated roots effectively checked internal extension of the disease for the duration of the experiment. In no case where the external growth was removed had the pathogen penetrated the root as far as the other side of the inspection hole although in the early months of the experiment the entire root surface within the holes became covered by superficial growth between the weekly treatments. The maximum penetration within those parts of the treated roots in the inspection holes was 4 inches, with an average of 1.74 inches in the young trees and 0.86 inch in the old trees. This greatly contrasts with the control roots, where the rhizomorphs were allowed to grow. In these, death was found in the young trees to extend beyond the inspection holes up to 19 inches with an average of 3.65 inches; in the old trees the maximum was 66 inches and the average 22.92 inches. The difference is illustrated by comparing *Figures* 2 and 3 (treated) with *Figures* 4 and 5 (control).

## OBSERVATIONS

When the inspection holes were dug, one month after inoculation, mycelium was already present on about half the roots. After a further month it was present on all except five roots of the old trees. In most cases the growth became very profuse, covering the entire surface of the roots. On the control roots much of this investing mycelium disappeared after twelve to sixteen weeks, leaving well defined rhizomorphs that had developed from it. The mycelium, at first white, became yellow after two to four weeks; the rhizomorphs, within three to six weeks, were orange or rust coloured. This colouration, when encountered in the field, is often attributed to the adsorption of pigments from the soil, especially in lateritic soil, but in this case such an explanation is difficult to accept since the mycelium within the inspection holes was not in contact with the soil. Although diffusion or translocation of pigments might have occurred from the soil at the edge of the pit, it is more likely that the pigment was produced by the fungus as it aged. Moreover, pigment is often found in old cultures.

TABLE 1. ROOT DISEASE EXPERIMENT. DETAILS OF ROOTS, INOCULA AND RESULTS OF INOCULATION AFTER ONE YEAR, WHEN THE SURFACE GROWTH OF *Fomes lignosus* WAS SCRAPED OFF

*5-year-old trees*

| Root | Root diameter at point of inoculation (inches) | Inoculum volume (cubic inches) | Length of penetration from outer end of hole (inches) | Length of penetration from inner end of the hole (inches) |
|---|---|---|---|---|
| 1 | 1.75 | 48 | 4.0 | 0.0 |
| 2 | 2.0 | 31 | 0.0 | 0.0 |
| 3 | 1.5 | 48 | 0.0 | 0.0 |
| 4 | 1.25 | 42 | 0.0 | 0.0 |
| 5 | 1.0 | 59 | 1.0 | 0.0 |
| 6 | 1.0 | 39 | 2.9 | 0.0 |
| 7 | 1.0 | 31 | 3.0 | 0.0 |
| 8 | 0.75 | 18 | 0.0 | 0.0 |
| 9 | 0.75 | 22 | 4.0 | 0.0 |
| 10 | 0.75 | 18 | 2.5 | 0.0 |
| Mean | 1.18 | 35.6 | 1.74 | 0.0 |

*25-year-old trees*

| | | | | |
|---|---|---|---|---|
| 1 | 2.0 | 61 | 0.0 | 0.0 |
| 2 | 2.0 | 53 | 0.8 | 0.0 |
| 3 | 1.5 | 31 | 2.5 | 0.0 |
| 4 | 1.5 | 21 | 0.0 | 0.0 |
| 5 | 2.5 | 59 | 0.0 | 0.0 |
| 6 | 1.0 | 49 | 0.0 | 0.0 |
| 7 | 1.0 | 39 | 1.8 | 0.0 |
| 8 | 1.0 | 29 | 2.0 | 0.0 |
| 9 | 0.75 | 18 | 0.0 | 0.0 |
| 10 | 0.75 | 46 | 1.5 | 0.0 |
| Mean | 1.40 | 40.6 | 0.86 | 0.0 |

TABLE 2. ROOT DISEASE EXPERIMENT. DETAILS OF ROOTS, INOCULA AND RESULTS OF INOCULATION AFTER ONE YEAR, WHEN THE SURFACE GROWTH OF *Fomes lignosus* WAS NOT SCRAPED OFF

| Root | Root diameter at point of inoculation (inches) | Inoculum (volume) (cubic inches) | Length of superficial mycelium from inoculum | | Length of penetration from inoculum | | Length of penetration from outer end of hole (inches) | Length of penetration from inner end of hole (inches) |
|---|---|---|---|---|---|---|---|---|
| | | | Inwards (inches) | Outwards (inches) | Inwards (inches) | Outwards (inches) | | |
| *5-year-old trees* | | | | | | | | |
| 1 | 2.0 | 53 | 66 | 54 | 27 | 54 | 16.0 | 7.5 |
| 2 | 1.0 | 21 | 54 | 12 | 12 | 0 | 3.0 | 0.0 |
| 3 | 1.5 | 24 | 42 | 10 | 13 | 0 | 8.5 | 2.5 |
| 4 | 2.0 | 39 | 50 | 6 | 23 | 0 | 22.5 | 19.0 |
| 5 | 1.5 | 18 | 36 | 18 | 5 | 5 | 0.0 | 0.0 |
| 6 | 1.25 | 49 | 48 | 7† | 24 | 7† | 1.5 | 0.0 |
| 7 | 1.0 | 24 | 54 | 18 | 6 | 0 | 2.5 | 0.0 |
| 8 | 0.75 | 12 | 56 | 14 | 15 | 0 | 14.5 | 7.5 |
| 9 | 0.75 | 24 | *Mycel.* disappeared | | 0 | 0 | 0.0 | 0.0 |
| 10 | 1.0 | 32 | 48 | 24 | 11 | 8 | 1.25 | 0.0 |
| Mean | 1.28 | 29.6 | 45.4 | 16.3 | 13.6 | 7.4 | 6.98 | 3.65 |
| *25-year-old trees* | | | | | | | | |
| 1 | 1.5 | 29 | 24 | 3 | 9 | 0 | 0.0 | 0.0 |
| 2 | 2.0 | 31 | 56 | 33 | 51 | 14 | 41.0 | 33.0 |
| 3 | 2.0 | 40 | 30 | 10 | 16 | 0 | 10.7 | 3.7 |
| 4 | 1.25 | 58 | 102 | 60 | 87 | 45 | 72.0 | 66.0 |
| 5 | 1.25 | 46 | 40 | 9 | 26† | 0 | 11.5 | 4.5 |
| 6 | 1.0 | 46 | 60 | 60 | 50 | 48 | 38.0 | 30.0 |
| 7 | 0.75 | 18 | 12* | 10* | 4 | 0 | 0.0 | 0.0 |
| 8 | 0.75 | 44 | 84 | 36† | 50 | 36 | 40.0 | 33.0 |
| 9 | 0.75 | 39 | 54 | 12† | 48 | 12 | 41.0 | 30.0 |
| 10 | 1.0 | 18 | 54 | 12† | 46 | 12 | 36.0 | 29.0 |
| Mean | 1.23 | 36.9 | 51.6 | 24.5 | 38.7 | 16.7 | 29.02 | 22.92 |

* = traces of rhizomorphs only present on roots
† = roots disintegrated beyond the distance shown

A record of the amount of growth found in the inspection holes of the treated roots is presented as a combined weekly average for young and old trees in *Figure 1*. Eighteen weeks after inoculation the rate of growth decreased rapidly, doubtless due to the exhaustion of food material in the original inoculum.

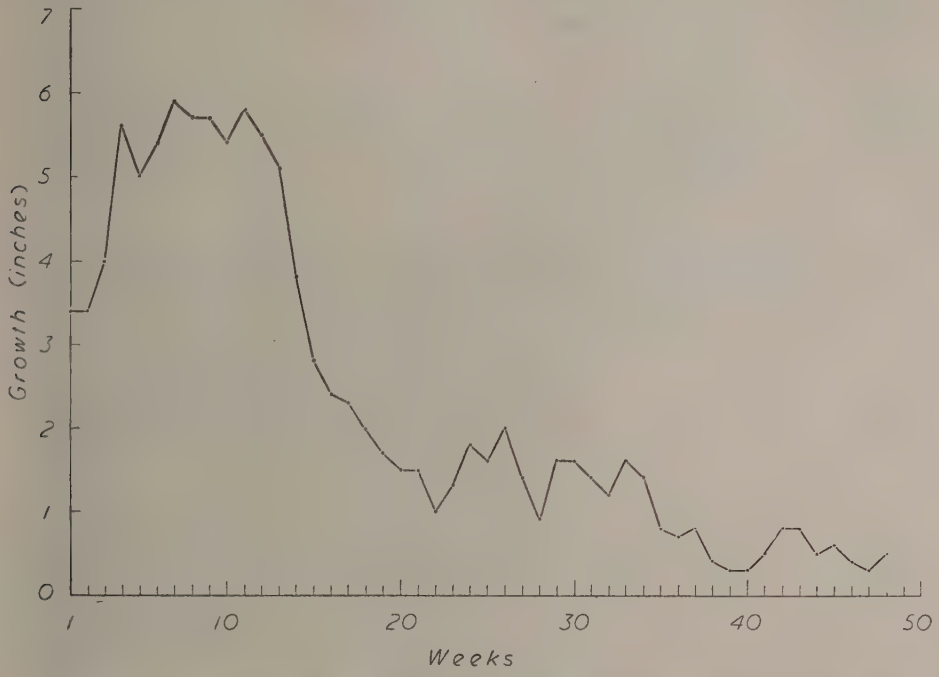


*Figure 1. The average weekly growth of Fomes lignosus in the inspection holes.*

Four and a half months after inoculation, that is about three months after the rhizomorphs had covered the root surface within the inspection holes, the bark of four untreated roots of the old trees and that of one of the young trees had been killed by the fungus within the holes. After six months these figures had doubled.

At the termination of the experiment (at the end of one year) all the inocula were found to be partially or completely disintegrated. In no case could the pathogen be isolated from them. Although a few retained their form, the inside was found to be either reduced to a soft black rot or converted into a mass of humus which, in many cases, was permeated by rubber feeding roots (*Figures* 6 and 7). Feeding roots had also penetrated dead sections of some of the inoculated roots (*Figures* 8 and 9). The condition of the inocula and their lack of viability is in marked contrast to previous experimental results. In an earlier experiment on the length of viability of the three major root pathogens (*F.lignosus*, *F.noxius* and *Ganoderma pseudoferreum*), sections of infected roots were buried in a loose sandy loam in an area free from cover plants which was kept clean weeded. In root sections of a size similar to those used in the present experiment, *F.lignosus* was found viable for up to two years (ALTSON 1953a, 1953b). There is no doubt that cover plants hasten the rate of decay and in this instance the feeding roots of the rubber appear also to have aided the process.

The inocula used in this experiment were all of sufficient volume to be able to initiate an infection. The smallest one used, of 12 cubic inches, sufficed to cause partial death of a lateral root and penetration at the collar. The few failures may have been due to variability of the inocula or to some undetermined soil effect. In a previous inoculation experiment with young seedlings in a nursery ALTSON (1953c) reported that inocula of 5 cubic inches or more were necessary to assure initiating an infection. With progressively smaller inocula, the proportion of successful inoculations fall off. The smallest size that induced an infection was ¼ cubic inch. However, in his experiment the inocula were placed in contact with the tap root of young seedlings a little below the collar — an area known to be especially susceptible to infection. DE JONG (1933) reported infection of young seedlings with natural inocula of 3 cubic inches and with artificial inocula of 1.8 cubic inches.

When the control roots were examined at the termination of the experiment it was noticed that both the external rhizomorph growth and penetration of the wood was more extensive inwards from the inoculum than outwards towards the distal portion of the root. Growth and penetration inwards was approximately twice as great as outwards from the inoculum.

The mean penetration inwards from the inoculum in the control roots was 38.7 inches for the old trees and 13.6 inches for the young trees. The two figures differ significantly ($P<0.02$). This result might be attributable to different degrees of resistance, or to different amounts of food reserves within the roots. If the old roots contained greater quantities of reserve food materials and thus might be expected to give greater impetus to fungal growth, it would be reasonable to expect a comparable increase in the amount of external growth on the older roots. However, although the average external growth was greater on the old roots—51.6 inches against 45.4 inches—the difference was found to be not significant. There was no significant difference between the volumes of inocula used for the two groups. It does not seem likely that this difference in penetration could be attributed to soil factors, thus it would appear to result from the young roots having a greater degree of resistance. This view is substantiated by the greater degree of barrier formation observed in the roots of young trees.

A contrary opinion was expressed by NAPPER (1932a), who believed that as the tree grows older it becomes more resistant to attack. The apparent contradiction might be resolved by supposing that, whilst the tree is growing rapidly and vigorously—up to, say, its twelth year—its resistance increases, and thereafter decreases.

Although both NAPPER (1932a) and DE JONG (1933) thought that the fungus did not penetrate through wounds, there is clear evidence from the present experiment that it does. Examples are shown in *Figures 10* to *13*, the presence of the fungus having been confirmed in all cases by its isolation in pure culture. The wounds had been caused when the root systems were first exposed in preparation for the experiment. Lenticels, also, were more readily invaded than the intact bark (*Figures 14* to *17*), while infected branch rootlets in addition facilitated entry of the fungus (*Figure 18*). In all cases the identity of the fungus was confirmed by isolating it from the lesions.

The cracking and fissuring of the bark at the collar might be regarded as a sort of self-wounding. The greater susceptibility of such bark to infection by *F.lignosus* than other parts of the root system is confirmed by this experiment. In four of the young

trees it was found that there was extensive death of the bark and some penetration of the wood at the collar, although the lateral roots along which the rhizomorphs had grown were free of all traces of penetration for two to three feet proximal to the collar (*Figure 19*).

Casual field observations confirm the statement of DE JONG (1933) that the rot caused by *F.lignosus* will sometimes stop without treatment. Detailed examination of the roots from this experiment show that host resistance may be effected in three ways.

Primary resistance may come from the cork cambium, which reacts to invasion by proliferating to form patches of thick corky bark which tend to crack longitudinally and to flake off (*Figure 20*). This proliferation is also noticeable when infection occurs at the lenticels and is accompanied by proliferation of parenchyma within the lenticels (*Figure 17*, p). If the fungus penetrates to the wood, wound barriers may be formed. These however, are unlikely to be successful where a vigorous inoculum is promoting the infection, each attempt at wound barrier formation being defeated by further penetration behind it from the advancing external rhizomorphs. When, however, the initial inoculum potential is too low for the infection to reach a self propagating level, then the wound barriers may succeed in completely sealing off the lesion. *Figure 21* shows evidence of a wound barrier having formed and been broken through, and the pathogen then having been halted successfuly by a second barrier. Other examples of barrier formation are shown in *Figures 10* to *13*. The final stage of the host reaction is callus formation commencing from the edges of the lesion (*Figures 12* and *22* to *25*). From some of the partly healed infections the pathogen could still be isolated, but in others it had died out and been replaced by *Diplodia* and other saprophytes.

Evidence from the present experiment supports the view that the fungus penetrates into the wood of the infected root through medullary rays (DE JONG 1933). In most cases of partial penetration the infection had stopped at, or just short of, the centre of the root. This was sometimes so even when a root was dead along one side for many feet, the other side (with no rhizomorph) remaining healthy.

## DISCUSSION

The most striking finding of this experiment is that, by preventing external growth of *F.lignosus*, the internal spread of infection is virtually halted. This observation greatly encourages the belief that chemical treatments may come to play an important part in control measures. If the collar and proximal part of the lateral roots can be kept free of rhizomorphs by chemical means, the host reaction may be able to cut off the disease, which will decay to nothing.

The experiment has incidentally provided evidence of wounds and lenticels being more readily invaded than is the intact bark. It has also indicated the changes in activity of the fungus in the pieces of naturally infected wood used as inocula. Defensive mechanisms of the host are also indicated.

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*September*

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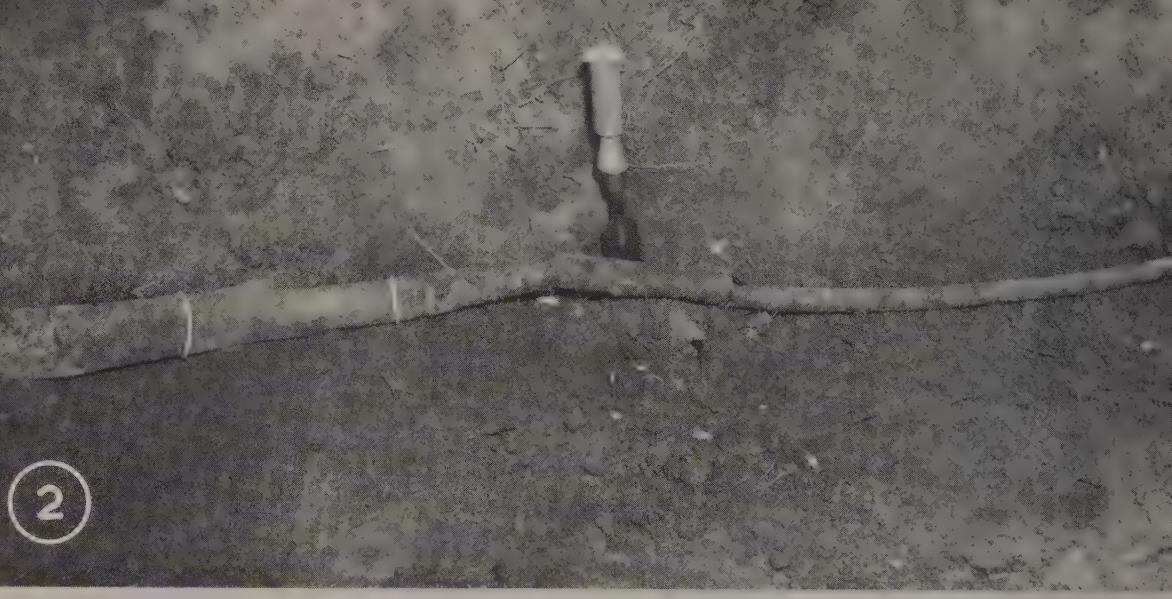
In the following *Figures* the white lines on the entire roots, and the black lines on sectioned roots, denote the position of the inspection hole.



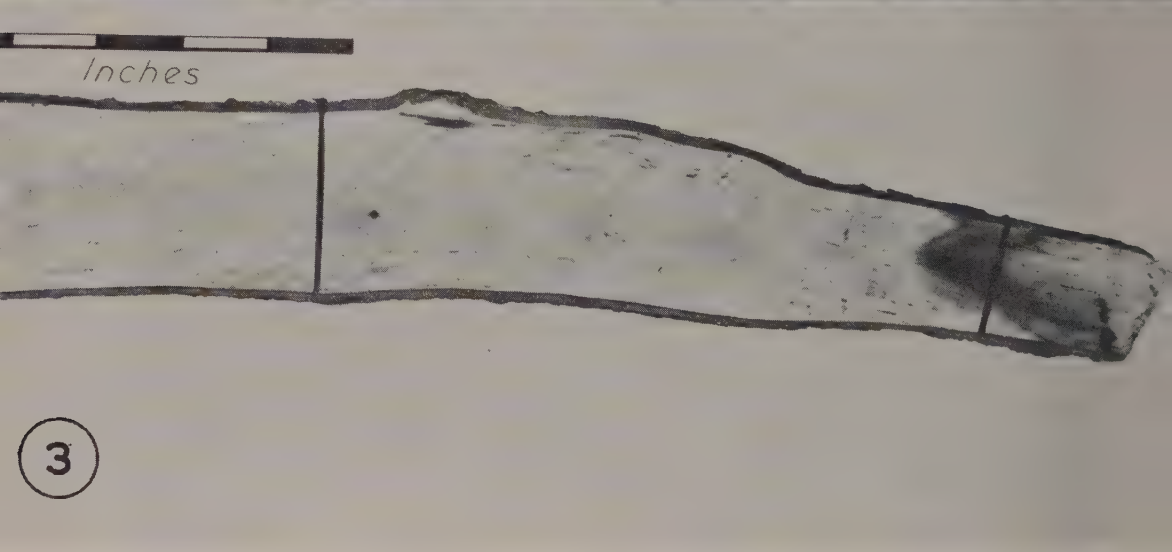







*Figure 2. A treated root of an old tree, dead up to the distal end of the hole and breaking into pieces at the site of the inoculum (marked with a chisel), the rot being so far advanced.*

*Figure 3. The same root in section showing the limit of the rot, which ends about an inch within the inspection hole at the distal end.*

*Figure 4. A control root of an old tree showing the position of the inspection hole and of the inoculum (marked with a parang). Rhizomorphs have grown right up to the collar of the tree.*

*Figure 5. The same root in section showing complete death of the root inside the inspection hole and for a further distance towards the tree, with death of the outer bark extended considerably further still.*

Figures 6 & 7. Inocula which are almost rotted away and penetrated by feeding roots of rubber.

Figure 8. A root of an old tree, the dead part penetrated by numerous feeding roots.

Figure 9. The same root in section showing the rot, with feeding roots on the surface and healthy parts on either side.

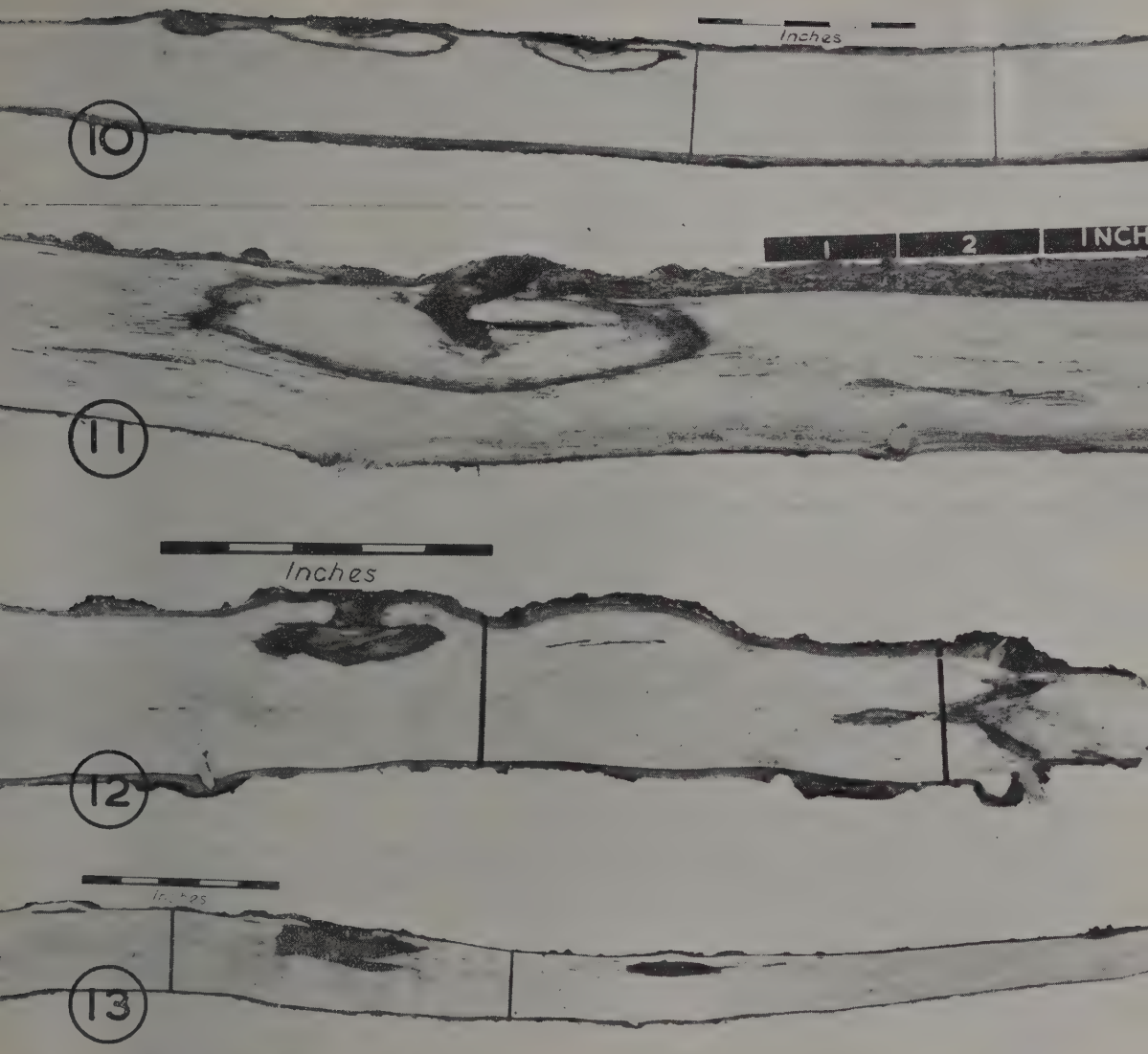



*Figure 10.* Section of a root of a young tree, showing two sites of wound penetration and barrier formation.

*Figure 11.* Section of a root of a young tree showing penetration through a deep wound, limited by formation of a barrier.

*Figure 12.* Section of a root of a young tree showing wound penetration where the wound is being closed by callus formation.

*Figure 13.* Section of a root of a young tree showing two sites of wound penetration. The one on the right is completely healed and covered by callus, whilst that on the left is partially healed and not yet covered.

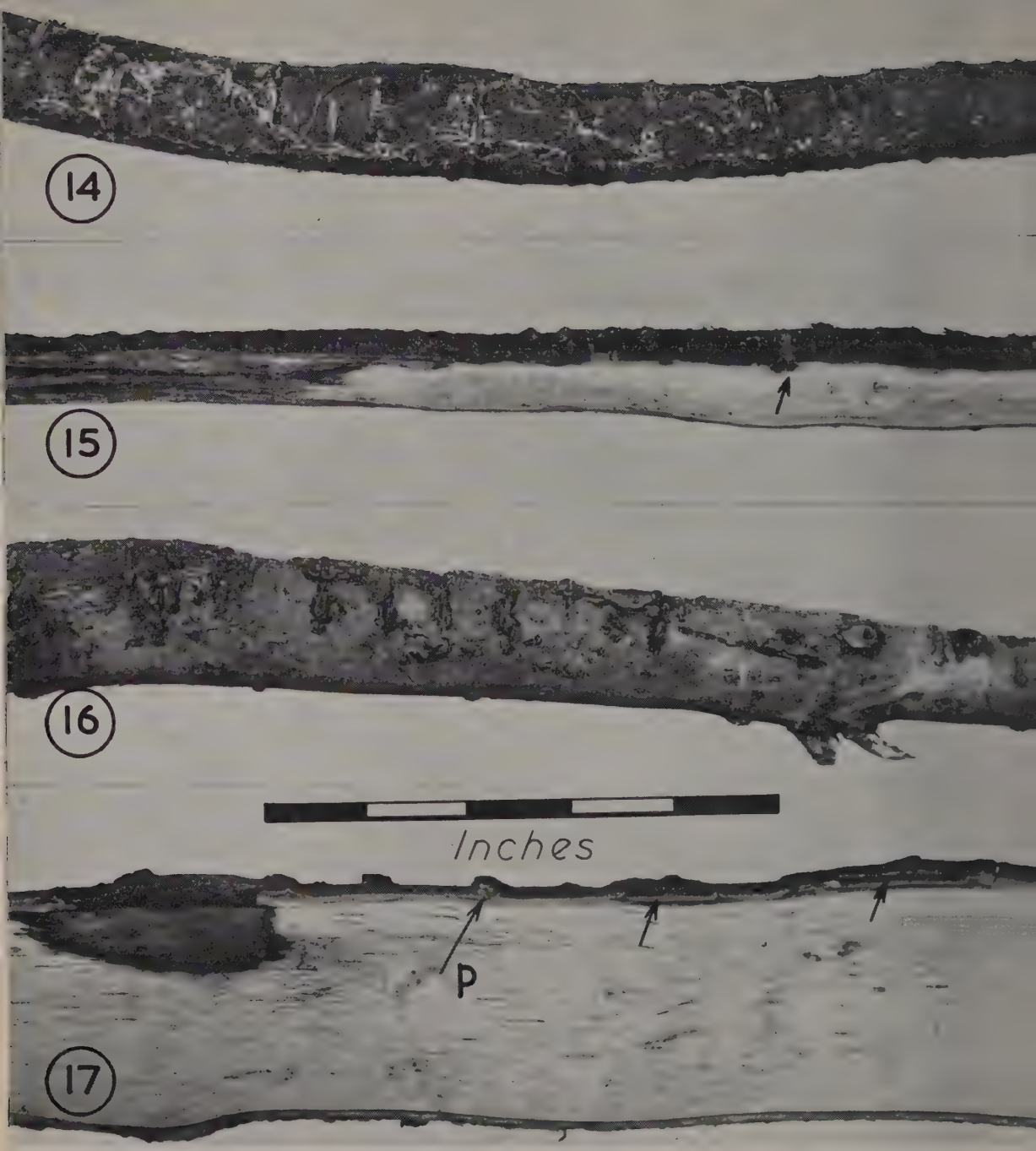



*Figure 14.* A control root of an old tree showing enlarged lenticels and rhizomorphs running along them.

*Figure 15.* The same root in section showing dead bark (seen black) beneath the lenticels (indicated by the arrow).

*Figure 16.* An infected root of a young tree showing enlarged lenticels.

*Figure 17.* The same root in section showing thick layers of dead bark (seen black) beneath the lenticels, with healthy bark beneath. The letter p shows the proliferation of parenchyma within the lenticels.

*Figure 18.* Section of a root of a young tree showing penetration through a small infected branch root (indicated by the arrow).

*Figure 19.* The bole of an untreated young tree scraped to show areas of collar penetration and death (seen black) with healthy areas (seen white) around. The inoculated root, which was not penetrated for some length near the collar, has been cut off.

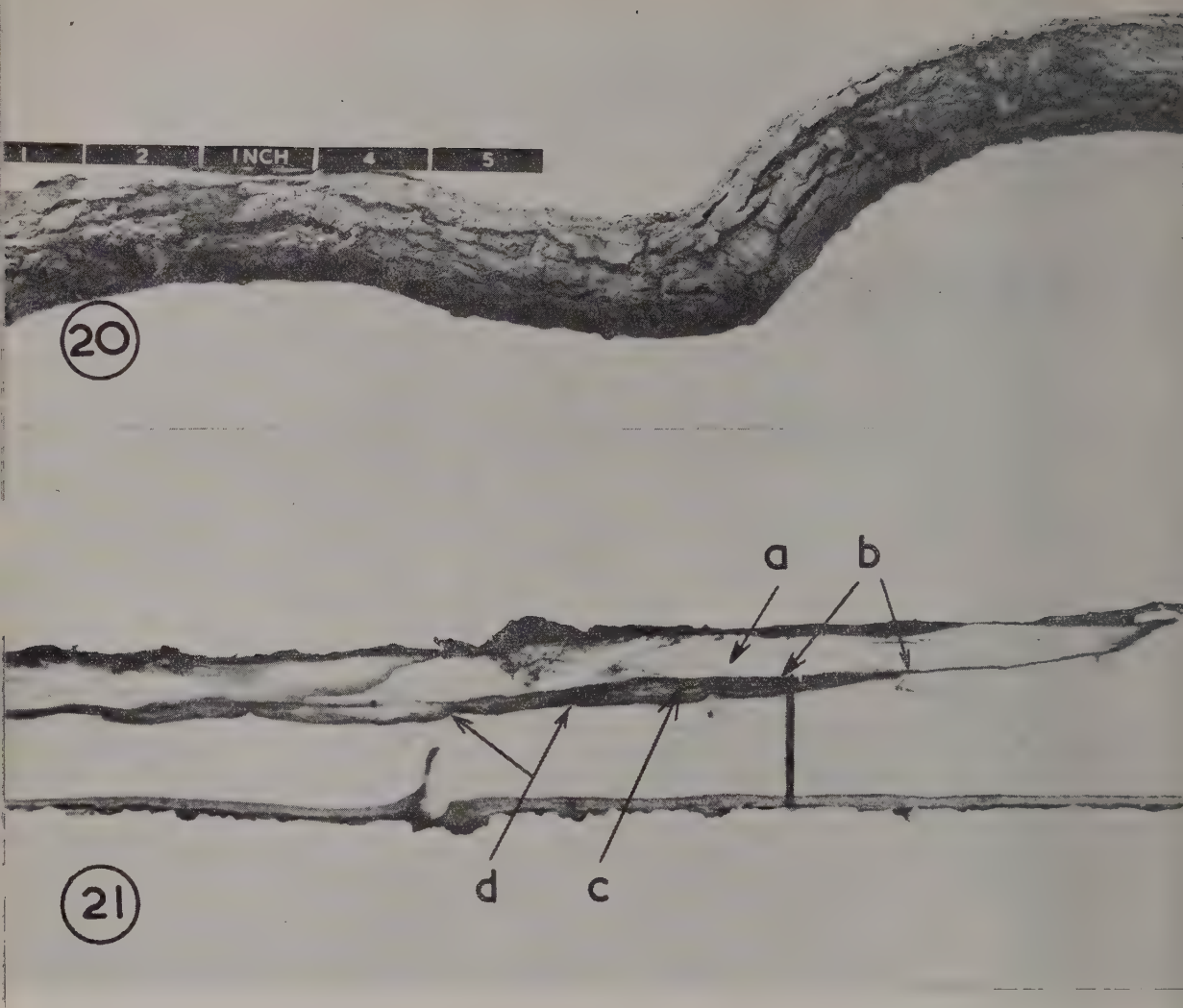






*Figure 20. Section of a root of a young tree showing proliferation and death of the outer bark, which is being sloughed off.*

*Figure 21. Section of a root of a young tree showing how penetration of the fungus has been limited by barrier formation. The white part at the top a shows an advanced soft rot which was initially checked by a brown barrier b, the black part below shows a hard rot, of recent origin c, the fungus having broken through the original barrier, whilst a second barrier is now forming d.*

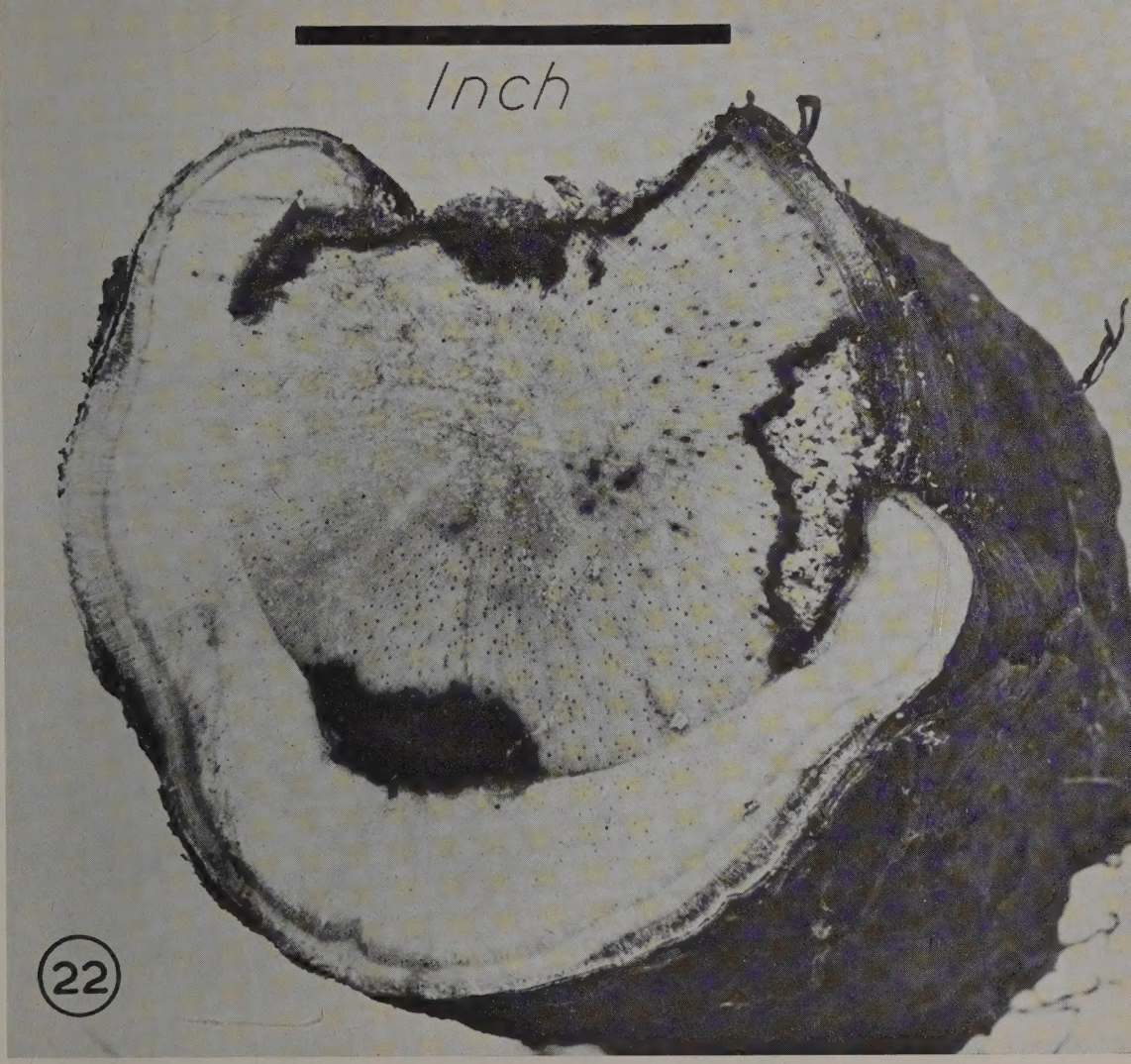



*Figure 22. The cross section of a root of a young tree showing extensive infection and regeneration by callus growth.*

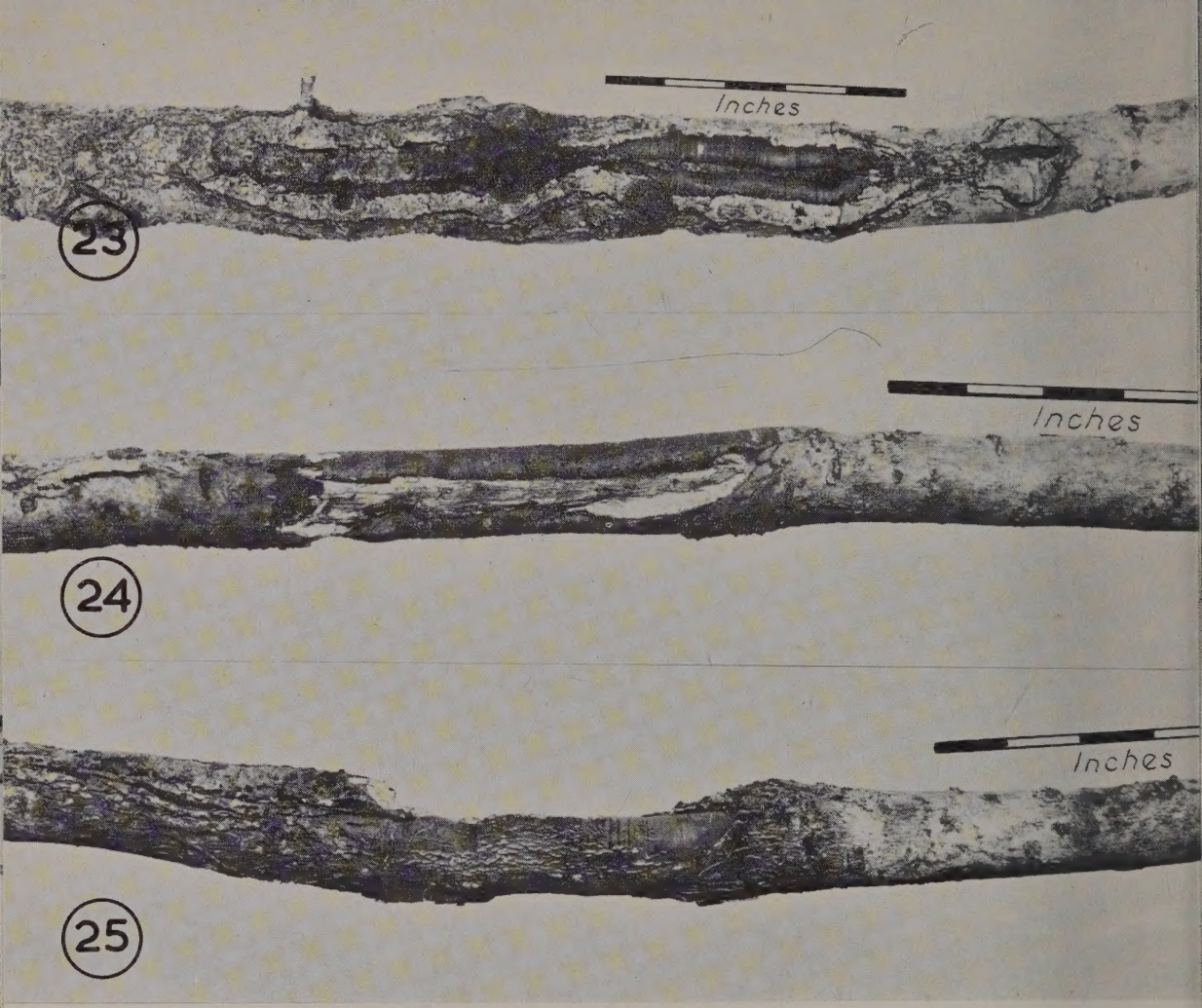



*Figure 23. A root of a young tree showing healing of a large lesion.*

*Figure 24. A root of a young tree showing healing of a part, where extensive penetration had originally killed more than three quarters of the cross section of the root.*

*Figure 25. The other side of the same root shown in Figure 24.*

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